

FORM PTO-1390 (REV. 12-2001)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 113122.120
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
			Unassigned 10/031478
INTERNATIONAL APPLICATION NO. PCT/AU00/00886	INTERNATIONAL FILING DATE 21 July 2000	PRIORITY DATE CLAIMED 23 July 1999	
TITLE OF INVENTION Beta-Amyloid Peptide Inhibitors			
APPLICANT(S) FOR DO/EO/US Barnham, K.; McCarthy, T.; Pallich, S.; Matthews, B.; Cherny, R.			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.</p> <p>4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <p>a. <input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</p> <p>b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</p> <p>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</p> <p>6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).</p> <p>a. <input type="checkbox"/> is attached hereto.</p> <p>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p>a. <input checked="" type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</p> <p>b. <input checked="" type="checkbox"/> have been communicated by the International Bureau.</p> <p>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p>d. <input type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).</p> <p>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p>Items 11 to 20 below concern document(s) or information included:</p> <p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.</p> <p>14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>15. <input type="checkbox"/> A substitute specification.</p> <p>16. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.</p> <p>18. <input checked="" type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</p> <p>19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</p> <p>20. <input checked="" type="checkbox"/> Other items or information: International Search Report International Preliminary Examination Report Application Data Sheet</p>			

U.S. APPLICATION NO. (if known, see 37 CFR 1.53) Unassigned 10/031478		INTERNATIONAL APPLICATION NO. PCT/AU00/00886		ATTORNEY'S DOCKET NUMBER 113122.120	
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21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY	
				\$ 1040.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	44 - 20 =	24	x \$18.00	\$ 432.00	
Independent claims	3 - 3 =	0	x \$84.00	\$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ \$280.00	\$
TOTAL OF ABOVE CALCULATIONS =				\$1602.00	
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				+	
SUBTOTAL =				\$ 801.00	801.00
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED =				\$	801.00
				Amount to be refunded:	\$
				charged:	\$ 801.00

a. ☐ A check in the amount of \$ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 08-0219 in the amount of \$ 801.00 to cover the above fees.
 A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
 overpayment to Deposit Account No. 08-0219. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card
 information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR
 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Hollie L. Baker, Esq.
 Hale and Dorr LLP
 60 State Street
 Boston, MA 02109

Hollie L. Baker
 SIGNATURE

Hollie L. Baker
 NAME

31,321
 REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(DO/EO/US)

Applicant: Barnham *et al.*

Art Unit: Unassigned

Serial No.: Unassigned

Examiner: Unassigned

Filing Date: Herewith

Title: Beta-Amyloid Peptide Inhibitors

BOX PCT

Assistant Commissioner for Patents
Washington, DC 20231

CERTIFICATION UNDER 37 C.F.R. § 1.10

I hereby certify that this correspondence is being deposited on 1/18/02 with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 "Express Mail" Mailing Label EVO3824560905 in an envelope addressed to Box PCT, Assistant Commissioner for Patents, Washington, DC 20231.

1/18/02
Date of signature and
of mail deposit

Sharon Matthews
Sharon Matthews

PRELIMINARY AMENDMENT

Dear Sir:

Prior to the substantive examination of the above-identified application, kindly amend the application as follows:

Amendments to the Specification:

Please add the following Abstract after page 45 of the Specification:

ABSTRACT

The present invention relates to compounds which inhibit the binding of metal ions to a region in the N-terminal loop of the β -amyloid peptide which includes a cluster of histidine residues. In addition, the invention relates to pharmaceutical

compositions including these compounds as the active agent, and to methods of treatment involving the administration of these compounds. The compounds of the invention are useful in the treatment of Alzheimer's Disease and other amyloid-related conditions. In a first aspect the present invention provides a compound which interacts with the β -amyloid peptide in such a way that the N-terminal loop of the peptide (amino acid residues 1-15) is blocked or destabilised, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop. Preferably the compound inhibits binding of Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.

Amendments to the Claims:

Please amend claims 3, 6-7, 11, 15, 19, 21, 25-27, 29-30, and 35-41, and add new claims 43-44 as follows. A clean version of the amended claims and the new claims is submitted in accordance with 37 C.F.R. § 1.121(c)(1)(i). A copy of the marked up amended claims in accordance with 37 C.F.R. § 1.121(c)(1)(ii) and a clean version of the entire set of pending claims in accordance with 37 C.F.R. § 1.121(c)(3) are attached hereto.

3. (Amended) A compound according to claim 1 which has a conformation and polarity such that it binds to at least one histidine residue in the N-terminal loop, selected from the group consisting of His6, His13 and His14.
6. (Amended) A compound according to claim 1, which also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11.
7. (Amended) A compound according to claim 1, which has acidic groups which interact with one or more of the His residues in the N-terminal loop.
11. (Amended) A compound according to claim 1, which is an organic molecule, a peptide or a metal complex.

15. (Amended) A compound according to claim 1, which comprises, or is conjugated to, a targeting moiety, forming an inhibitor-targeting moiety complex.
19. (Amended) A compound according to claim 15, in which the targeting moiety targets the compound to a site defined by residues 15-21 of the β -amyloid peptide.
21. (Amended) A compound according to claim 15, in which the inhibitor-targeting moiety complex is able to penetrate the blood-brain barrier.
25. (Amended) A method according to claim 22, in which the compound also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glul.
26. (Amended) A method according to claim 22, in which the compound inhibits binding of Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.
27. (Amended) A method according to claim 22, in which the compound has overall hydrophobic character.
29. (Amended) A compound which inhibits the binding of metal ions to the N-terminal loop of the β -amyloid peptide, wherein the compound is obtained by a method according to claim 22.
30. (Amended) A composition comprising a compound according to claim 1, together with a pharmaceutically-acceptable carrier.
35. (Amended) A method according to claim 31, in which the compound also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glul.
36. (Amended) A method according to claim 31, in which the compound inhibits binding of Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.

37. (Amended) A method according to claim 31, in which the compound is a complex of Mn, Fe, Co, Ni, Cu, Zn, Ru, Pd, Ag, Cd, Pt, Au, Rh or Hg, with the proviso that the compound is not haemin or haematin.
38. (Amended) A method according to claim 31, in which the compound comprises, or is conjugated to, a targeting moiety.
39. (Amended) A method according to claim 38, in which the targeting moiety targets the compound to a site defined by residues 15-21 on the β -amyloid peptide.
40. (Amended) A method according to claim 31, in which the inhibition of binding of one or more metal ions to the β -amyloid peptide occurs *in vivo*.
41. (Amended) A method of prevention, treatment or alleviation of Alzheimer's disease, which method comprises the step of administering a compound according to claim 1 to a subject in need of such treatment.
43. (New) A composition comprising a compound according to claim 29, together with a pharmaceutically acceptable carrier.
44. (New) A method of prevention, treatment or alleviation of Alzheimer's disease, which method comprises the step of administering a pharmaceutical composition according to claim 30 to a subject in need of such treatment.

REMARKS

Upon entry of this Preliminary Amendment, claims 1-44 will be pending. The foregoing amendments to the claims were made to eliminate multiple dependency and typographical errors, and to clarify that an inhibitor-targeting moiety complex is formed in claim 15. The specification has been amended to insert after the claims the abstract that appears on the cover page of the published international application. No new matter has been introduced by these amendments. Early and favorable examination on the merits is respectfully requested.

No fees are believed to be due in connection with this correspondence.
However, please charge any payments due or credit any overpayments to our Deposit
Account No. 08-0219.

The Examiner is encouraged to telephone the undersigned in order to expedite
the prosecution of the instant application.

Respectfully submitted,
HALE AND DORR LLP



Hollie L. Baker
Reg. No. 31,321

January 18, 2002

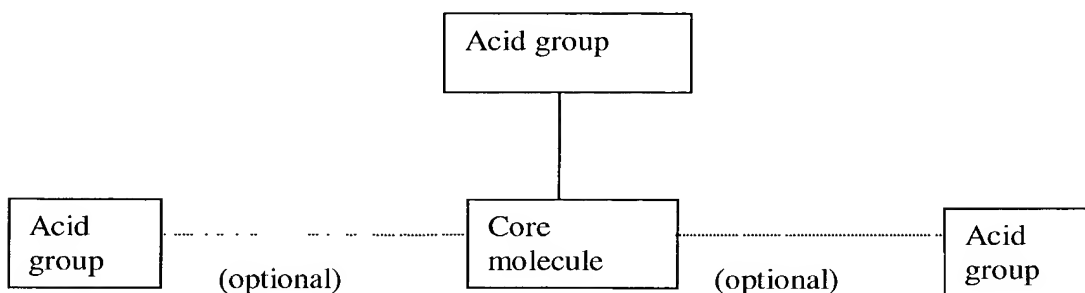
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27. (Amended) A method according to [any one of] claim[s] 22 [to 26], in which the compound has overall hydrophobic character.
29. (Amended) A compound which inhibits the binding of metal ions to the N-terminal loop of the β -amyloid peptide, wherein the compound is obtained by a method according to [any one of] claim[s] 22 [to 28].
30. (Amended) A composition comprising a compound according to [any one of] claim[s] 1 [to 21 or claim 29], together with a pharmaceutically-acceptable carrier.
35. (Amended) A method according to [any one of] claim[s] 31 [to 34], in which the compound also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11.
36. (Amended) A method according to [any one of] claim[s] 31 [to 35], in which the compound inhibits binding of Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.
37. (Amended) A method according to [any one of] claim[s] 31 [to 36], in which the compound is a complex of Mn, Fe, Co, Ni, Cu, Zn, Ru, Pd, Ag, Cd, Pt, Au, Rh or Hg, with the proviso that the compound is not haemin or haematin.
38. (Amended) A method according to [any one of] claim[s] 31 [to 37], in which the compound comprises, or is conjugated to, a targeting moiety.
39. (Amended) A method according to claim 38, in which the targeting moiety targets the compound to [the] a site defined by residues 15-21 on the β -amyloid peptide.
40. (Amended) A method according to [any one of] claim[s] 31 [to 39], in which the inhibition of binding of one or more metal ions to the β -amyloid peptide occurs *in vivo*.
41. (Amended) A method of prevention, treatment or alleviation of Alzheimer's disease, which method comprises the step of administering a compound according to [any one of] claim[s] 1 [to 21 or a pharmaceutical composition according to claim 30] to a subject in need of such treatment.

Clean Version of Pending Claims Under 37 C.F.R. § 1.121(c)(3)

1. A compound which interacts with the β -amyloid peptide in such a way the N-terminal loop of the peptide (amino acid residues 1-15) is blocked or destabilised, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop.
2. A compound according to claim 1 which inhibits binding Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.
3. (Amended) A compound according to claim 1 which has a conformation and polarity such that it binds to at least one histidine residue in the N-terminal loop, selected from the group consisting of His6, His13 and His14.
4. A compound according to claim 3, which binds to at least two histidine residues in the N-terminal loop.
5. A compound according to claim 4, which binds to at least three histidine residues in the N-terminal loop.
6. (Amended) A compound according to claim 1, which also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11.
7. (Amended) A compound according to claim 1, which has acidic groups which interact with one or more of the His residues in the N-terminal loop.
8. A compound according to claim 7, represented as follows:



wherein the core molecule has a conformation and polarity such that the acid group(s) interact with one or more of His6, His13 and His14.

9. A compound according to claim 8, in which the acid group is selected from the group consisting of CO_2H , PO_3H_2 , SO_3H , OSO_3H_2 , and OPO_3H_2 .
10. A compound according to claim 9, which is a molecule with one to three carboxylic acid groups, the length of the molecule being such that it can be received within the N-terminal loop, and such that at least one carboxyl group is in proximity to at least one of the histidine residues.
11. (Amended) A compound according to claim 1, which is an organic molecule, a peptide or a metal complex.
12. A compound according to claim 9, which is not a metal complex.
13. A compound according to claim 9, which has overall hydrophobic character.
14. A compound according to claim 10, which is able to penetrate the blood-brain barrier.
15. (Amended) A compound according to claim 1, which comprises, or is conjugated to, a targeting moiety, forming an inhibitor-targeting moiety complex.
16. A compound according to claim 15, in which the targeting moiety is selected from the group consisting of polypeptides, nucleic acids, carbohydrates, lipids, β -amyloid ligands, antibodies, and dyes.
17. A compound according to claim 15, in which the targeting moiety has a hydrophobic region which interacts with the tail of the β -amyloid peptide.
18. A compound according to claim 17, in which the targeting moiety comprises a fatty acid molecule.

19. (Amended) A compound according to claim 15, in which the targeting moiety targets the compound to a site defined by residues 15-21 of the β -amyloid peptide.

20. A compound according to claim 17, in which the targeting moiety is a peptide which comprises a sequence which corresponds to that of residues 15-21 of the β -amyloid peptide.

21. (Amended) A compound according to claim 15, in which the inhibitor-targeting moiety complex is able to penetrate the blood-brain barrier.

22. A method of selecting or designing a compound which inhibits the binding of metal ions to the N-terminal loop of the β -amyloid peptide, which method comprises the steps of

(i) selecting or designing a compound which has a conformation and polarity such that it binds to at least one amino acid in the N-terminal loop selected from the group consisting of His6, His 13 and His14; and

(ii) testing the compound for the ability to inhibit binding of metal ions to the N-terminal loop of the β -amyloid peptide.

23. A method according to claim 22, in which the compound binds to at least two histidine residues in the N-terminal loop.

24. A method according to claim 23, in which the compound binds to at least three histidine residues in the N-terminal loop.

25. (Amended) A method according to claim 22, in which the compound also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11.

26. (Amended) A method according to claim 22, in which the compound inhibits binding of Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.

27. (Amended) A method according to claim 22, in which the compound has overall hydrophobic character.
28. A method according to claim 27, in which the compound is able to penetrate the blood-brain barrier.
29. (Amended) A compound which inhibits the binding of metal ions to the N-terminal loop of the β -amyloid peptide, wherein the compound is obtained by a method according to claim 22.
30. (Amended) A composition comprising a compound according to claim 1, together with a pharmaceutically-acceptable carrier.
31. A method of inhibiting the binding of one or more metal ions to the β -amyloid peptide, or of inhibiting the aggregation of β -amyloid peptide, which method comprises the step exposing the peptide to a compound which blocks or destabilises the N-terminal loop of the peptide, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop.
32. A method according to claim 31, in which the compound has a conformation and polarity such that it binds to at least one histidine residue in the N-terminal loop of the β -amyloid peptide, selected from the group consisting of His6, His13 and His14.
33. A method according to claim 32, in which the compound binds to at least two histidine residues in the N-terminal loop.
34. A method according to claim 33, in which the compound binds to at least three histidine residues in the N-terminal loop.
35. (Amended) A method according to claim 31, in which the compound also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glul1.

36. (Amended) A method according to claim 31, in which the compound inhibits binding of Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.
37. (Amended) A method according to claim 31, in which the compound is a complex of Mn, Fe, Co, Ni, Cu, Zn, Ru, Pd, Ag, Cd, Pt, Au, Rh or Hg, with the proviso that the compound is not haemin or haematin.
38. (Amended) A method according to claim 31, in which the compound comprises, or is conjugated to, a targeting moiety.
39. (Amended) A method according to claim 38, in which the targeting moiety targets the compound to a site defined by residues 15-21 on the β -amyloid peptide.
40. (Amended) A method according to claim 31, in which the inhibition of binding of one or more metal ions to the β -amyloid peptide occurs *in vivo*.
41. (Amended) A method of prevention, treatment or alleviation of Alzheimer's disease, which method comprises the step of administering a compound according to claim 1 to a subject in need of such treatment.
42. A method of prevention, treatment or alleviation of Alzheimer's disease, which method comprises inhibiting the binding of one or more metal ions to the β -amyloid peptide, or inhibiting the aggregation of β -amyloid peptide, by the method of claim 40.
43. (New) A composition comprising a compound according to claim 29, together with a pharmaceutically acceptable carrier.
44. (New) A method of prevention, treatment or alleviation of Alzheimer's disease, which method comprises the step of administering a pharmaceutical composition according to claim 30 to a subject in need of such treatment.

BETA-AMYLOID PEPTIDE INHIBITORS

The present invention relates to compounds which inhibit the binding of metal ions to the N-terminal region of the β -amyloid peptide. In addition, the present invention relates to pharmaceutical compositions including these compounds as the active agent, and to methods of treatment involving the administration of these compounds. The compounds of the invention are useful in the treatment of Alzheimer's disease.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is characterised by the presence of distinctive lesions in the victim's brain. These brain lesions include abnormal intracellular filaments called neurofibrillary tangles, and extracellular deposits of amyloid in senile, or amyloid, plaques. Amyloid deposits are also present in the walls of cerebral blood vessels of Alzheimer's patients.

The major constituent of amyloid plaques has been identified as a 4 kilodalton peptide (39-43 residues) called β -amyloid peptide ($A\beta$) (Glennner and Wong, 1984). Diffuse deposits of $A\beta$ peptides are frequently observed in normal adult brains, whereas Alzheimer's disease brain tissue is characterised by more compacted, dense-core β -amyloid plaques. These observations suggest that $A\beta$ deposition precedes, and contributes to, the destruction of neurons that occurs in Alzheimer's disease. In further support of a direct pathogenic role for AD, β -amyloid has been shown to be toxic to mature neurons both in culture and *in vivo* (Yanker et al., 1989).

Natural $A\beta$ is derived from proteolysis from a much longer protein known as the amyloid precursor protein (APP) (Kang, J et al, 1987). The APP gene maps to chromosome 21, thereby providing an explanation for the β -amyloid deposition seen at an early age in individuals with Down's syndrome, which is caused by trisomy of chromosome 21.

5 suggest that the aggregated peptide has redox properties and can generate reactive oxygen species, which attack enzymes and possibly cell membranes, causing neurotoxicity (Markesbery, W.R. 1997). Antitioxidants are known to protect against A β -induced toxicity.

10 A β has been shown to bind copper and iron in
stoichiometric amounts, with the associated formation of
reactive oxygen species such as peroxides and hydroxide
radicals, which are possible sources of the neurotoxicity
(Bush et al., 1998). While the formation of peroxide in
15 post-mortem samples of Alzheimer's disease brain has been
observed, there was little peroxide formation in control
tissue (Cherny et al., 1998). The peroxidase activity
observed in the samples of Alzheimer's disease brain was
abolished when treated with certain chelators (Cherny et
20 al., 1998). The formation of reactive oxygen species was
accompanied by a reduction in the valence state of the
metal, ie Cu(II) to Cu(I) and Fe(III) to Fe(II) (Atwood et
al., 1998a). Reactive oxygen species can also lead to free
radical formation on the A β peptide, which leads over time
25 to covalent cross-linking of the A β peptides (Bush et al.,
1998). In addition, a number of metal ions, including Zn,
Ni and Cu, have been shown to induce aggregation of A β
(Atwood et al., 1998b). When brain tissue from both control
and Alzheimer's disease-affected subjects was treated with
30 chelators which are specific for zinc and copper, there was
greatly enhanced solubilisation of A β , with an increase of
up to 700%, suggesting that zinc and copper play a role in
the assembly of the A β deposits (Cherny et al., 1998).

Histidine residues have been implicated in the binding of metal ions to A β peptides. For instance rat A β 1-40, in which His13 is mutated to Arg, does not aggregate, nor does A β 1-40 treated with diethyl

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pyrocarbonate, which binds to the imidazole nitrogen of histidine (Atwood et al., 1998). Subsequently to the priority date of this application, it was reported that three histidine residues in the N-terminal hydrophilic region of human A β provide primary metal binding sites, and that the solubility of the complex between metal and A β depends on the mode of metal binding. The authors proposed that Cu²⁺ would protect A β against Zn-induced aggregation by competing with zinc ions for binding sites on the histidine residues (Miura et al., 2000).

In contrast, we propose that inhibition of binding of zinc, copper and/or iron to the A β peptide will have significant therapeutic value in the treatment of Alzheimer's disease.

It has been reported that certain tetrapyrroles, especially certain porphyrin and phthalocyanine compounds inhibit conversion of normal, protease-sensitive prion protein (PrP^{sen}) to the protease-resistant form (PrP^{res}) which is implicated in the pathogenesis of transmissible spongiform encephalopathies (TSEs) such as Creutzfeldt-Jacob disease (Caughey et al., 1998), and that three of these compounds inhibited TSE disease *in vivo* (Priola et al., 2000). However, both metal-free and metal-complexed tetrapyrroles were active, and the authors considered that the mechanism of action involved direct interaction between the compound and the infectious agent. Although the authors speculated that the compounds might also be useful in the treatment of non-prion mediated amyloid-related conditions, such as Alzheimer's disease or Type II diabetes, this was no more than speculation (Priola et al., 2000). Moreover, all of the compounds disclosed have multiple substitutions on the tetrapyrrole ring, whereas the tetrapyrrole compounds of the present invention are preferably substituted only on one of the rings.

It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of

these documents forms part of the common general knowledge in the art, in Australia or in any other country.

SUMMARY OF THE INVENTION

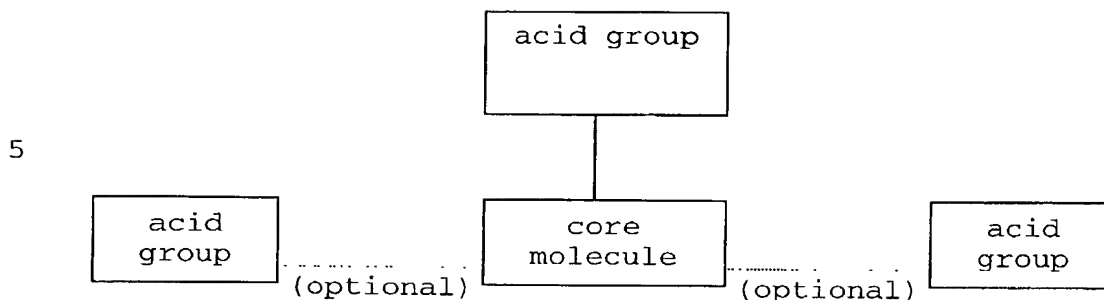
5 The present inventors have now found that zinc and copper bind predominantly to a region in the N-terminal loop of A β which includes a cluster of histidine residues. This finding provides the basis for the rational design or selection of inhibitors of the binding of zinc, copper
10 and/or iron to A β .

 Accordingly, in a first aspect the present invention provides a compound which interacts with the β -amyloid peptide in such a way that the N-terminal loop of the peptide (amino acid residues 1-15) is blocked or
15 destabilised, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop.

 Preferably the compound inhibits binding of Cu²⁺, Zn²⁺ and Fe³⁺ ions, but not Mg²⁺ or Ca²⁺ ions.

20 Preferably the compound has a conformation and polarity such that it binds to at least one, more preferably at least two, and more preferably three histidine residues in the N-terminal loop, selected from the group consisting of His6, His13 and His14. More
25 preferably the compound also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11.

 The compound may have acidic groups which interact with one or more of the His residues in the N-terminal
30 loop. For example, the compound may be represented as follows:



10 wherein the core molecule has a conformation and polarity such that the acid group(s) interact with one of more of His6, His13 and His14. The acid may be any acid group, including, but not limited to, CO_2H , PO_3H_2 , SO_3H , OSO_3H_2 , OPO_3H_2 and the like.

15 The compound may be a molecule with one to three carboxylic acid groups, the length of the molecule being such that it can be received within the N-terminal loop, and such that at least one carboxyl group is in proximity to at least one of the histidine residues. Without wishing
20 to be bound by theory, we believe that it is likely that such molecules will have a molecular mass in the region of 2000 Daltons.

The compound may be an organic molecule, a peptide or a metal complex. In this aspect of the invention, however,
25 it is preferred that the compound is not a metal complex. Preferably the compound has overall hydrophobic character. More preferably the compound is able to penetrate the blood-brain barrier.

In a particularly preferred embodiment of the
30 invention, the inhibitor compound comprises, or is conjugated to, a targeting moiety.

The term "targeting moiety" as used herein refers to a functional group which will specifically interact with the β -amyloid peptide. That is, the inhibitor compound
35 includes or is covalently linked to a targeting moiety which will specifically bind to or associate with the β -amyloid peptide. Suitable targeting moieties include,

but are not limited to, polypeptides, nucleic acids, carbohydrates, lipids, β -amyloid ligands, antibodies, dyes and the like. In a preferred embodiment the targeting moiety has a hydrophobic region which interacts with the tail of the β -amyloid peptide. For example, the targeting moiety may include a fatty acid molecule.

Preferably the targeting moiety targets the compound to the site defined by residues 15-21 of the β -amyloid peptide. The targeting moiety may be a peptide which comprises a sequence which corresponds to that of residues 15-21 of the β -amyloid peptide. More preferably the inhibitor-targeting moiety complex is able to penetrate the blood-brain barrier.

In a second aspect, the invention provides a method of selecting or designing a compound which inhibits the binding of metal ions to the N-terminal loop of the β -amyloid peptide, which method comprises the steps of

(i) selecting or designing a compound which has a conformation and polarity such that it binds to at least one, more preferably at least two and more preferably three amino acids in the N-terminal loop, selected from the group consisting of His6, His 13 and His14; and

(ii) testing the compound for the ability to inhibit binding of metal ions to the N-terminal loop of the β -amyloid peptide.

Preferably the compound inhibits binding of Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.

Preferably the compound has a conformation or polarity such that it also binds to at least one amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11. Preferably the compound also has overall hydrophobic character. More preferably the compound is able to penetrate the blood-brain barrier.

In a third aspect, the invention provides a compound which inhibits the binding of metal ions to the N-terminal loop of the β -amyloid peptide, wherein the compound is

obtained by a method according to the second aspect of the invention.

In a fourth aspect, the invention provides a composition comprising a compound according to the first or the third aspects of the present invention, together with a pharmaceutically acceptable carrier. Methods and pharmaceutical carriers for preparation of pharmaceutical compositions are well known in the art, as set out in textbooks such as Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Easton, Pennsylvania, USA.

The compounds of the present invention may be formulated into pharmaceutical compositions, and administered in therapeutically effective doses. The term "therapeutically effective dose" means a dose which results in the inhibition of natural binding of metal ions to the N-terminal loop of the β -amyloid peptide. The pharmaceutical compositions may be administered in a number of ways, including, but not limited to, orally, subcutaneously, intravenously, intraperitoneally and intranasally. The most appropriate dose and route of administration will be dependent on the age and general state of health of the subject to be treated, and will be at the discretion of the attending physician. This dose can be readily ascertained by one skilled in the art, using well-known techniques.

In a fifth aspect, the invention provides a method of inhibiting the binding of one or more metal ions to the β -amyloid peptide, or inhibiting the aggregation of β -amyloid peptide, which method comprises the step of exposing the peptide to a compound which blocks or destabilises the N-terminal loop of the peptide, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop.

35 Preferably the compound has a conformation and polarity such that it binds to at least one, more preferably at least two, and more preferably three

5

10

20

site defined by residues 15-21 on the β -amyloid peptide.

30

35

The patient is monitored for clinical improvement, which may commence within as little as one week, but more probably may be observed at six weeks, and may take as long as 12 months. The normal clinical indices which are used in the monitoring of patients with the relevant condition are used. Where the treatment is prophylactic, the patient is monitored for signs of development of the condition. The attending clinician will be aware of the most suitable tests to use.

Where this method is to be used for prophylactic purposes, the subject is preferably one at increased risk of developing the condition. For example, the subject may have one or more family members with the condition, eg. familial Alzheimer's disease, or may have trisomy of chromosome 21 (Down's syndrome).

It will be clearly understood that, for the purposes of this aspect of the invention, the compound is not haemin.

In a seventh aspect, the invention provides a method of monitoring the efficacy of treatment according to the method of the invention, comprising the steps of obtaining a sample of a biological fluid for a patient undergoing treatment, and measuring the level of $A\beta$ in the sample, in which increased $A\beta$ levels compared to levels of $A\beta$ in a normal control sample are indicative of the efficacy of the treatment.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning. /

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a representation of the β -amyloid peptide showing a structured turn in the region of amino acid residues 15-21.

Figure 2 shows a model of Zn bound to the three histidine residues of $A\beta$ 1-40.

Figure 3 is a NMR spectrum showing the effect of Zn^{2+} binding to A β 1-28.

Figure 4 is a NMR spectrum showing the effect of Cu^{2+} binding to A β 1-28.

5 Figure 5 is a NMR spectrum showing the effect of addition of Cu^{2+} and cobalt complex to A β 1-28.

Figure 6 is a NMR spectrum showing the binding of the cobalt complex to A β 1-28.

10 Figure 7 is a Western blot showing results of brain tissue assays testing the ability of a range of metal compounds to solublilize A β deposits.

Figure 8 shows a model of the cobalt-corrin ring complex bound to A β 1-40.

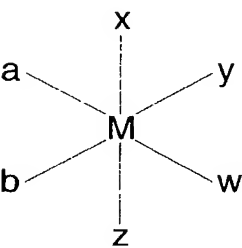
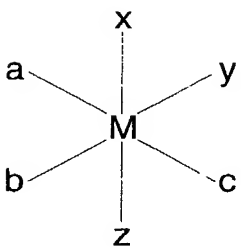
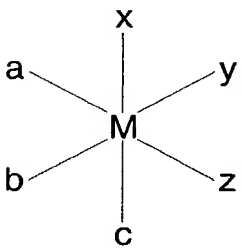
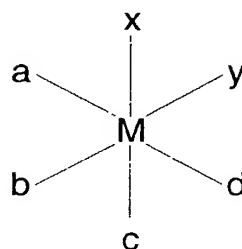
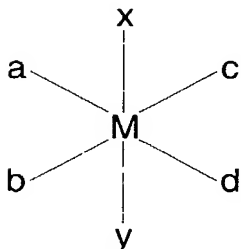
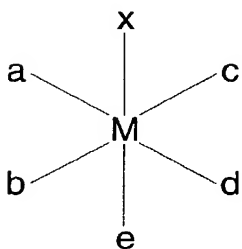
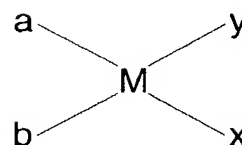
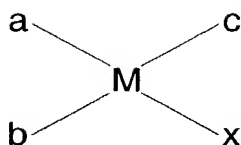
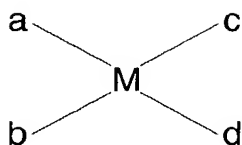
15 Figure 9 shows NMR spectra demonstrating the binding of the compound KJB001 to A β 1-28.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have developed three-dimensional structural information concerning the
20 N-terminal region of the β -amyloid peptide, and have identified a cluster of three histidine residues which constitute a binding site for metal ions. This information provides a rational basis for the development of compounds which inhibit the binding of metal ions to the N-terminal
25 loop of the β -amyloid peptide. Such inhibitors have the potential to inhibit aggregation of β -amyloid peptides and to reduce metal- induced neurotoxicity. Accordingly, these inhibitors are likely to have therapeutic value in the treatment of diseases such as Alzheimer's disease.

30 Using the structural information provided by the inventors, the general principles of drug design can be applied by persons skilled in the art to produce compounds which preferentially bind to at least one of the histidine residues in the N-terminal loop (ie His6, His13 or His14),
35 and inhibit the binding of metal ions to the N-terminal loop of the amyloid peptide.

Preferred inhibitors within the context of the present invention include metal complexes which can exchange or bind functional moieties such as histidine. Preferably the metal complex is capable of binding between
5 1 and 3, preferably 2 or 3, histidine residues of the N-terminal loop of the β -amyloid peptide. The complex may bind to other residues in addition to the histidine residues. Metal ions capable of binding to the imidazole nitrogen of histidine include Mn, Fe, Co, Ni, Cu, Zn, Ru,
10 Pd, Ag, Cd, Pt, Au, Rh and Hg . Complexes of these metals are expected to be predominantly four coordinate tetrahedral (distorted tetrahedral)/square planar complexes, or six coordinate octahedral (or distorted octahedral) complexes. In the case of the four coordinate
15 complexes they could react with the β -amyloid peptide to replace ligands; alternatively, by binding to His residues as well as to their initial ligand(s) their coordination number is increased to 5 or 6. Five coordinate complexes with either a trigonal bipyramid or square pyramid
20 configuration may also be used. Examples of suitable complexes are shown below.

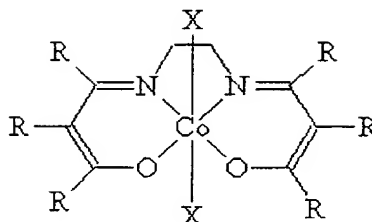


In these formulae:

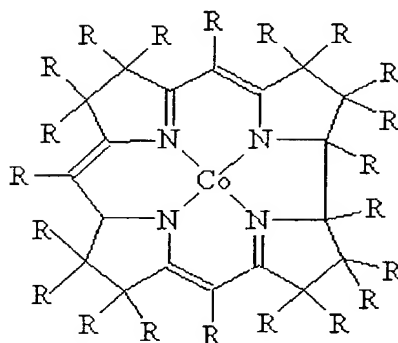
- (i) a, b, c, d and e are non-leaving groups,
 5 preferably chelating groups including Schiff bases, porphyrin rings, macrocycles, polyamino-carboxylates, heterocyclic aromatic groups such as 2,2'-bipyridine and 1,10-phenanthroline, peptides, nucleobases, or chelating ligands in which one of the donor atoms is a phosphine
 10 phosphorus atom;
- (ii) M is a metal; and
- (iii) w, x, y and z are leaving groups (ie those groups which will be replaced by histidine and possibly other residues when the metal complex reacts with the
 15 β -amyloid peptide), and include halogens, amines, ammonia, pyridyls, imidazoles, nucleobases, peptides, H_2O/OH , carboxylic acids, phosphates, sulfates, nitrate, triflate, or alkoxides.

Those skilled in the art will recognise that the appropriate combination of non-leaving and leaving groups will be dependent on the identity of the metal.

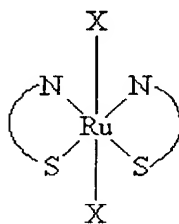
Metal complexes which have the potential to bind to histidine residues are described in WO 97/21431 and WO 96/18402, the entire contents of which are incorporated herein by this reference. Other examples of complexes which may act as inhibitors are as follows:



I



II



III

In formulae I, II and III, the X and R groups may be any suitable leaving or targeting groups. For example, X and R may be the same or different, and include, without being limited to, the group consisting of ammine; amine; peptide; halogen (chloride, fluoride or iodide); nucleobase; imidazole; H₂O; hydrogen; saturated or unsaturated alkyl, alcohol, or carboxylate of 1 to 10 carbon atoms; aromatic; or heterocycle of up to four rings.

Other preferred compounds include cobalt(II) phthalocyanine β -form; (S,S)-(+)-N-N'-bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexane diamino manganese (III)

chloride; iron(II) phthalocyanine bis(pyridine) complex; iron(III) phthalocyanine chloride; manganese(II) phthalocyanine; 5,10,15,20-tetraphenyl-21H, 23H-porphine manganese (III) chloride; chloro(pyridine) bis (dimethylglyoximate) cobalt (III); N-N'-bis(salicylidene)dianilino-cobalt(II); cis-bis(2-2'-bipyridine)dichloro-ruthenium (II) hydrate; and cobalt(acacen) (NH₃)₂Cl, in which (H₂acacen represents bis(acetylacetonate)-ethylenediamine).

10 Compounds which are designed or selected according to
the methods of the invention may be tested for inhibitory
activity by any suitable assay procedure. Assays to
determine the binding of metal complexes to A β may be
performed by NMR or UV-Visible spectroscopy, or by ESR in
15 the case of paramagnetic metals. Assays are available for
measuring Cu/Fe reduction, hydrogen peroxide, hydroxyl
radical generation, and carbonyl group, all of which assess
the redox capacity of A β in the presence of Cu and Fe. *Ex*
vivo assays using *post mortem* brain tissue may also be
20 performed. These include measuring the amount of A β which
is solubilised and extracted in the presence of the
compound, and determining the quantity of peroxide formed
in *post mortem* brain tissue, as compared with control
tissue which is solubilised and extracted in the absence of
25 the compound. Suitable methods are described for example in
PCT/US99/05291 (WO99/45907).

The invention will now be described in detail by way of reference only to the following non-limiting examples and to the drawings.

30

35 The $^3J_{\text{NHCOH}}$ coupling constants were measured from a DQF-COSY spectrum or by using the INFIT module of XEASY to analyze NOESY spectra.

For $^3J_{\text{NHC}\alpha\text{H}}$ coupling constants measured from a DQF-COSY the appropriate rows were extracted from the spectrum, inverse Fourier transformed, zero filled to 32 K data points, and multiplied by a Gaussian window function prior to Fourier transformation. The dispersive peak shapes were simulated to take account of the effect of broad linewidths on small coupling constants, using an in-house program, COUPLING.

10 *Metal Binding Studies*

Metal binding studies were performed by titrating concentrated metal solutions (30 mM CuCl_2 , ZnCl_2 in water) into the peptide solutions described above. The displacement of bound Cu^{2+} from A β 1-28 by the Co(III) Schiff-base was performed by adding two equivalents of Cu^{2+} to A β 1-28, followed by one equivalent of Co(III) Schiff-base.

Brain Tissue Assays

20 Tissue selection

Post-mortem tissues, stored at -80°C , were obtained from the NH&MRC-supported Brain Bank at the University of Melbourne, together with accompanying histopathological and clinical data. AD was assessed according to CERAD criteria (Mirra et al, 1991). In order to examine the chemical architecture of the $\text{A}\beta$ deposition observed in non-AD aged brain, $\text{A}\beta$ immunohistochemistry was used to select age-matched control (AC) cases which did not fulfil the CERAD criteria, and in which amyloid deposition, if present, was detectable only in the form of diffuse plaques, but not neuritic plaques.

Preparation of compounds

Compounds were dissolved in DMSO and diluted in a PBS
35 mixture. Insoluble material was removed.

Sample preparation:

The cortical meninges were removed and gray matter (0.5 g) was homogenised using a DIAx 900 homogeniser (Heidolph & Co, Kelheim, Germany) for 3 x 30s periods at full speed, with a 30s rest between strokes, in 3 ml of ice-cold phosphate-buffered saline (PBS), pH 7.4, containing a mixture of protease inhibitors (BioRad, Hercules, CA), but without ethylene diamine tetraacetic acid (EDTA), or in the presence of test compounds or metal ions prepared in PBS. The homogenate samples were incubated for 24 h at room temperature. To obtain the PBS-extractable fraction, the homogenate was centrifuged at 100,000 x g for 30 min, the supernatant removed, and divided into 1 ml aliquots. Protein in a 1 ml supernatant sample was precipitated using 1:5 ice-cold 10% trichloroacetic acid (TCA), and pelleted by centrifugation at 10,000 x g for 20 mins. The pellet was prepared for PAGE by boiling for 10 min in Tris-tricine SDS-sample buffer containing 8% SDS, 10% mercaptoethanol and 8M urea. Total A β in the cortical samples was obtained by homogenizing in 1 ml PBS and boiling in sample buffer as described above.

Polyacrylamide gel electrophoresis (PAGE) and Western blotting

Tris-tricine PAGE was performed by loading samples on to 10-20% gradient gels (Novex, San Diego, CA), followed by transfer on to 0.2 mm nitrocellulose membrane (BioRad, Hercules, CA). The A β was detected using the following monoclonal antibodies: W02, which detects A β 40 and A β 42 at an epitope between residues 5 and 8; G210, which is specific for A β species which terminate at carboxyl residue 40; or G211, which is specific for A β species which terminate at carboxyl residue 42 (Ida et al, 1996), in conjunction with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG (Dako, Denmark), and visualised using chemiluminescence (ECL, Amersham Life Science, UK). Each gel included two or more lanes containing known quantities of synthetic A β (Keck Laboratory, Yale University New

Haven, CT) as reference standards. Blot films were scanned using a Relisys scanner with transparency adapter (Tech Information Systems, Taiwan) and densitometry performed using Image 1.6 software (NIH, Bethesda, MA). All samples were analysed at least twice, and gel loadings and dilutions adjusted to fit within the quantifiable region of the standard curve.

The efficiency of the TCA precipitation procedure was validated by testing samples of whole human serum diluted 1:10 to which had been added 2mg of synthetic A β 1-40 or A β 1-42. A β recovery was assessed by extracting the precipitate into SDS sample buffer and performing Western blot analysis, using synthetic A β standards as described above. Protein in the TCA pellet was estimated by resuspending the pellet in water and assaying the protein recovery using a BCA assay (Pierce, Rockford, IL). This indicated that the efficiency of protein and A β precipitation was approximately 90%. The efficiency of the 8M urea solubilization was found to be equivalent to that of formic acid in a parallel, blinded assay conducted independently. All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise indicated.

Example 1 Characterisation of A β peptides in aqueous solution

In aqueous solution there is little chemical shift difference between the amide and C^αH protons of Aβ1-28 compared with Aβ1-40, suggesting that both peptides are in a similar conformation. Comparisons of A1-28 and Aβ1-40 chemical shifts with random coil chemical shifts and the lack of NOE connectivities in the NOESY spectra indicate that both peptides are mostly in conformational exchange. However, there are some medium range NOE connectivities ($1 < |i-j| < 5$) observed in the region of residues 16-21 of the peptide (KLVFFA), suggesting that this region of the peptide has a structured turn. This is illustrated in Figure 1.

To determine the metal-binding site of A β 1-40 and A β 1-28 in aqueous solution, Zn²⁺ and Cu²⁺ were titrated into solutions of A β 1-40 and A β 1-28 at pH 6.9. All reactions were accompanied by significant precipitation. The NMR

	KJB001	Co(II) phthalocyanine β -form
25	KJB002	(S,S)-(+) -N-N'-bis(3,5-di-tert-butylsalicylidene)-1-2-cyclohexane diamino manganese (III) chloride
	KJB003	Haemin
	KJB004	Iron(II) phthalocyanine bis(pyridine) complex
	KJB005	Iron(III) phthalocyanine chloride
	KJB006	Manganese(II) phthalocyanine
30	KJB007	5,10,15,20-tetraphenyl-21H,23H-porphine manganese(III) chloride
	KJB008	Chloro(pyridine) bis(dimethylglyoximate)cobalt (III)
	KJB009	N-N'-bis(salicylidene)dianilino-cobalt(II)
35	KJB010	cis-bis(2-2'-bipyridine)dichloro-ruthenium (II) hydrate.

BR16805 [Co(acacen)(NH₃)₂]Cl (H₂acacen represents
bis(acetylacetone)ethylenediamine)

Table 1 shows the approximate concentration of each
5 compound used in the extraction.

Table 1

Compound	Concentration (μ mol/litre)
KJB001	0.0105
KJB002	4.41
KJB003	60.87
KJB004	3.816
KJB005	3.08
KJB006	111.5
KJB007	0.64
KJB008	1289.9
KJB010	300.9
BRI6805	1199.9
bathocuproine	2000

10 The results presented in Figure 7 clearly demonstrate
that some of the metal complexes have the ability to
solubilise A β deposited in the brains of Alzheimer's
disease patients. In particular, compound KJB001 showed
particularly strong solubilising activity, and compounds
15 KJB002, KJB005, KJB006, KJB007 and KJB010 also showed good
activity.

Example 4 Compounds designed to bind one or more of
the three histidine residues in A β

20 BRI7080, BRI7103 and BRI7104 are metal complexes of
aza-macrocycles of the following structures.

This compound has similar structures to BRI7080 and BRI7103, but in this case $M = \text{Co}^{3+}$ and $L = \text{Cl}$.

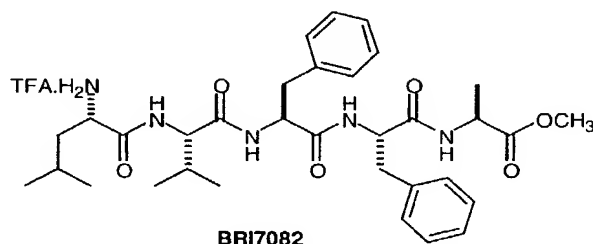
BRI7104 was prepared following the method of P-K. Chan and C-K. Poon, *J. C. S. Dalton Trans.*, 1976, 858-862.

Example 5 Histidine binding compounds conjugated with an A β -directing group.

For the second class of molecules we chose derivatives of the pentapeptide leucine-valine-phenylalanine-phenylalanine-alanine (LVFFA), designated herein as BRI7082 and BRI7077, to direct histidine binding compounds to A β .

BRI7106 is an example of a nicotine derivative conjugated to an A β -directing group. In this example the histidine-binding group is a small organic molecule rather than a metal complex, in contrast to most of the compounds described above. BRI7158 and BRI7159 are examples of metal complexes conjugated to an A β -directing group.

(a) BRI7082



BRI7082 was prepared via standard HBTU-mediated peptide coupling methodology; see M. Bodanszky and A. Bodanszky, *"The Practice of Peptide Synthesis"*, 2nd Edition, Springer-Verlag, 1994.

Mass Spectrum (APCI) m/z 610 [(MH-C₂H₂O₂F₃)⁺, 100%].

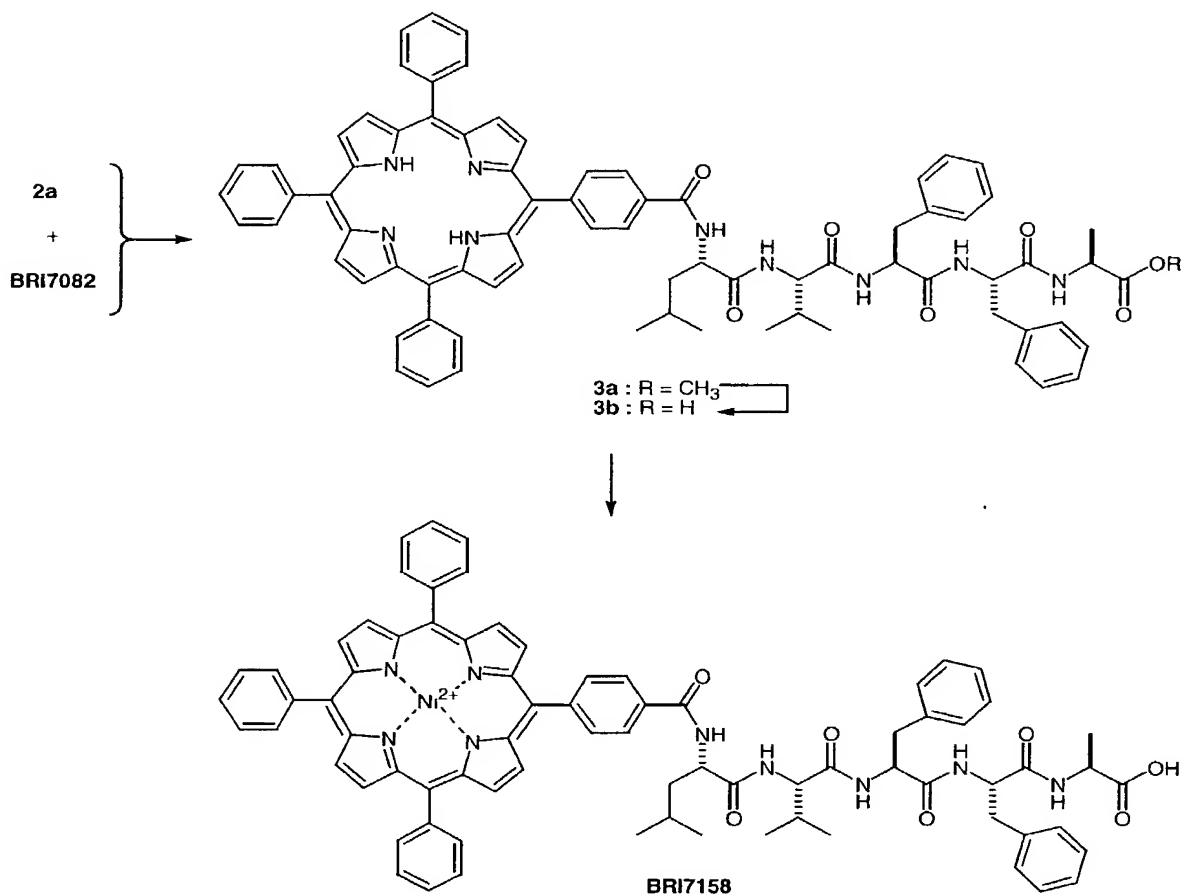
(b) BRI7106

This compound was synthesised according to Reaction Scheme 1:

Mass Spectrum (APCI) m/z 894 [(M+H)⁺, 10%], 892 [(M-H), 25%].

(c) Synthesis of BRI7158

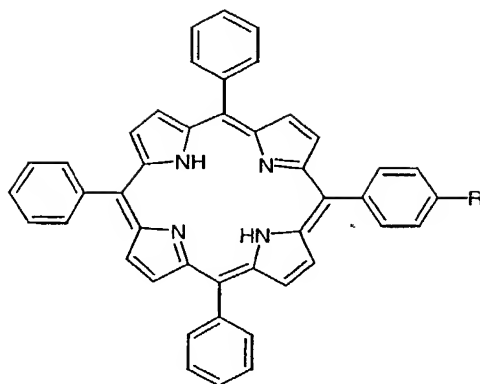
This compound was synthesised according to Reaction Scheme 2:



Scheme 2

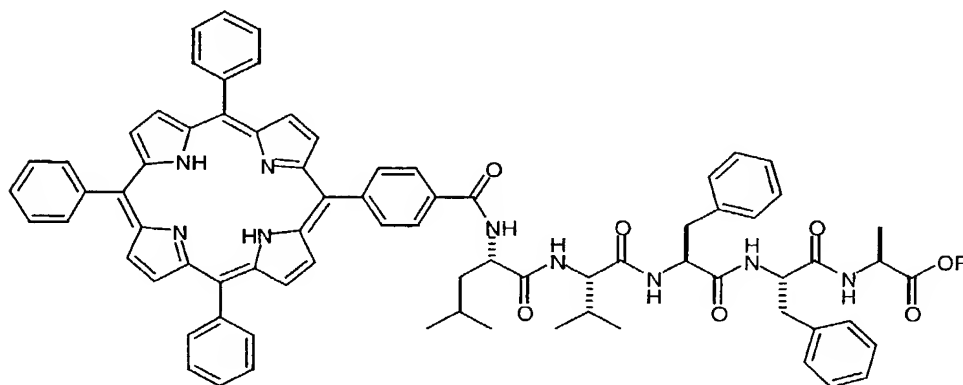
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Porphyrin 2a was prepared following the methods of K. Nakanishi *et al.*, *Heterocycles*, 1996, 42, 723-736 and D. A. James *et al.*, *Bioorg. Med. Chem. Lett.*, 1999, 9, 2379-2384.



2a : R = CO₂H

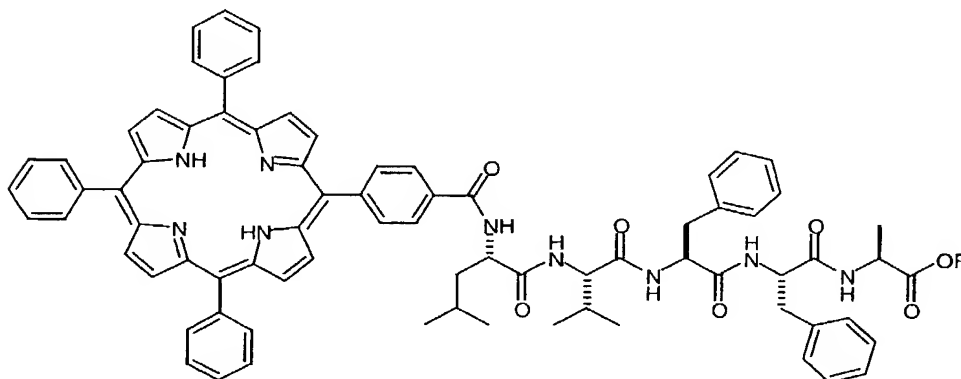
N, *N*-Diisopropylethylamine (0.10 ml, 0.57 mmol) was added to a magnetically stirred mixture of porphyrin 2a (118 mg, 0.18 mmol), HBTU (68 mg, 0.18 mmol), and BRI7082 (130 mg, 0.18 mmol) in dry DMF (4 ml) under an atmosphere of nitrogen. Stirring was continued at room temperature for 16 h, after which time, the crude reaction mixture was partitioned between ethyl acetate and brine. The separated organic layer was successively washed with 10% aqueous citric acid solution, saturated aqueous NaHCO₃ solution and brine before being dried (MgSO₄), filtered and concentrated under reduced pressure to afford compound 3a as a deep purple, crystalline solid.



3a : R = CH₃

Mass Spectrum (APCI) m/z 1250 $[(M+H)^+]$, 45%].

A solution of compound 3a (23.3 mg, 0.019 mmol, THF (0.5 ml), methanol (50 drops) and 2M aqueous NaOH solution (0.1 ml) was stirred at room temperature for 18 h. The crude reaction mixture was partitioned between ethyl acetate and water and the phases separated. The aqueous layer was acidified to pH 4 upon the addition of 10% aqueous citric acid solution and then extracted three times with ethyl acetate. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford compound 3b as a purple solid.



3b: R = H

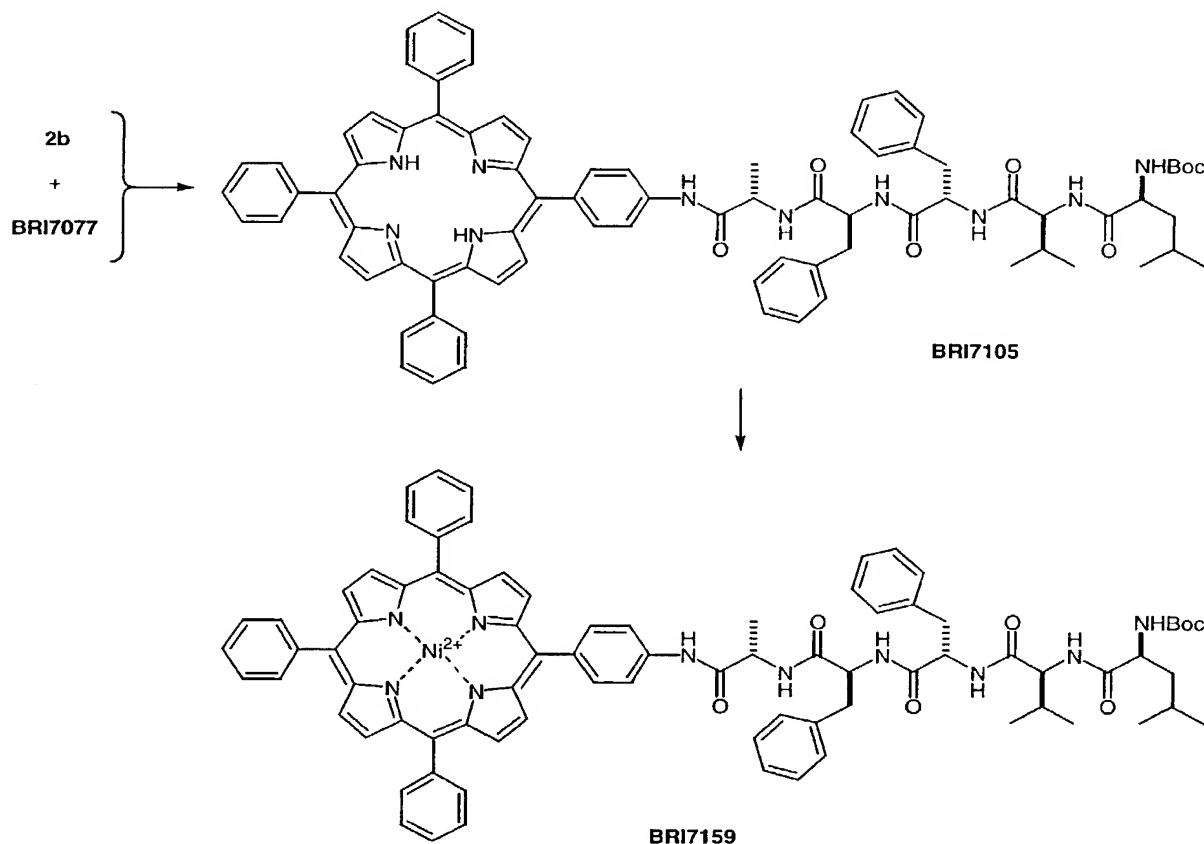
Mass Spectrum (APCI) m/z 1236 $[(M+H)^+]$, 30%].

A magnetically stirred mixture of compound 3b (17 mg, 0.014 mmol), $Ni(OAc)_2 \cdot 4H_2O$ (34.2 mg, 0.14 mmol), glacial acetic acid (0.73 ml) and dichloromethane (1.45 ml) was heated at reflux under an atmosphere of nitrogen for 18 h. The now crimson red reaction mixture was cooled to room temperature and partitioned between ethyl acetate and 10% aqueous $NaHCO_3$ solution. The separated aqueous phase was extracted three times with ethyl acetate before being dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a red solid. Subjection of this material to flash chromatography (silica, 10% methanol/dichloromethane

elution) afforded, after concentration of the appropriate fractions (R_f 0.30), BRI7158 as a red, crystalline solid. Mass Spectrum (ES) m/z 1291 [(M-H), <10%].

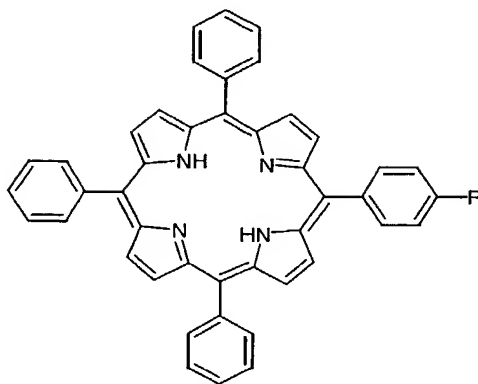
5 (d) Synthesis of BRI7105 and BRI7159

These compounds were synthesised according to Reaction Scheme 3:



Scheme 3

Porphyrin 2b was prepared following the method of W. J. Kruper, Jr. *et al.*, *J. Org. Chem.*, 1989, 54, 2753-2756.



2b : R = NH₂

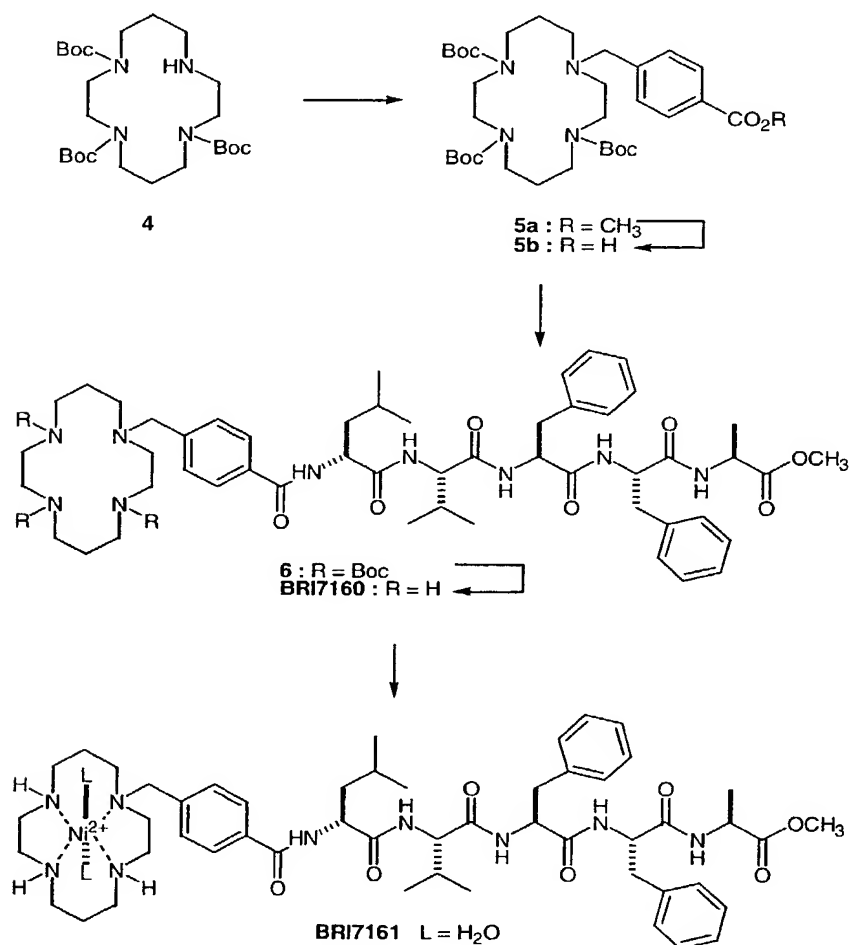
5 N, N-Diisopropylethylamine (0.21 ml, 1.18 mmol) was added at room temperature to a magnetically stirred mixture of porphyrin 2b (151 mg, 0.24 mmol), HBTU (216 mg, 0.58 mmol), and BRI7077 (200 mg, 0.29 mmol) in dry DMF (2 ml) under an atmosphere of nitrogen. Stirring was continued at 40°C for 40 h, after which time the crude reaction mixture was partitioned between ethyl acetate and brine. The separated organic layer was successively washed with 10% aqueous citric acid solution, 5% aqueous NaHCO₃ solution and brine before being dried (MgSO₄), filtered and concentrated under reduced pressure to afford a deep purple glass. Subjection of this material to flash chromatography (silica, 1% to 5% methanol/dichloromethane elution) gave, after concentration of the relevant fractions (R_f 0.35), BRI7105 as a purple, crystalline solid. Mass Spectrum (APCI) *m/z* 1307 [(M+H)⁺, <5%].

20 A magnetically stirred mixture of BRI7105 (47 mg, 0.04 mmol), Ni(OAc)₂·4H₂O (96 mg, 0.39 mmol), glacial acetic acid (2 ml) and dichloromethane (4 ml) was heated at reflux under an atmosphere of nitrogen for 18 h. The now crimson red reaction mixture was cooled to room temperature and partitioned between ethyl acetate and 10% aqueous

NaHCO₃ solution. The separated aqueous phase was extracted three times with ethyl acetate before being dried (Na₂SO₄), filtered and concentrated under reduced pressure to give a red solid. Subjection of this material to flash chromatography (silica, 10% methanol/dichloromethane elution) afforded, after concentration of the appropriate fractions (*R_f* 0.30), BRI7159 as a red, crystalline solid. Mass Spectrum (APCI) *m/z* 1385 [(M+Na)⁺, <5%].

10 (e) Synthesis of BRI7160 and BRI7161

The compounds were prepared according to Reaction Scheme 4:



Scheme 4

Compound 4 was prepared following the method of R. Guillard *et al.*, *Bull. Soc. Chim. Fr.*, 1996, 133, 65-73.

A magnetically stirred suspension of compound 4 (430 mg, 0.86 mmol), (4-bromomethyl) methyl benzoate (217 mg, 0.95 mmol), KHCO_3 (172 mg) and K_2CO_3 (174 mg) in dry acetonitrile (17 ml) was heated at reflux for 18 h under an atmosphere of nitrogen. The reaction mixture was cooled to room temperature then concentrated under reduced pressure to yield a straw coloured oil which was purified by flash chromatography (silica, 5% methanol/dichloromethane elution). Concentration of the appropriate fractions (R_f 0.38) afforded adduct 5a as a clear, colourless oil. Mass Spectrum (APCI) m/z 649 [$(M+H)^+$, 100%].

A mixture of compound 5a (120 mg, 0.19 mmol), 1M aqueous LiOH (5 ml) and THF (5 ml) was magnetically stirred at room temperature for 16 h. The crude reaction mixture was partitioned between ether and water and the separated aqueous layer was cooled to 0°C prior to acidification to pH 4 with 10% aqueous citric acid solution. Extraction of the aqueous layer with ethyl acetate, followed by drying of the organic fraction with MgSO_4 afforded, after concentration under reduced pressure, compound 5b as a viscous, colourless oil.

Mass Spectrum (APCI) m/z 633 [$(M-H)$, 100%].

N, N-Diisopropylethylamine (0.17 ml, 0.98 mmol) was added to a magnetically stirred mixture of compound 5b (167 mg, 0.26 mmol), HBTU (132 mg, 0.35 mmol), and BRI7082 (209 mg, 0.29 mmol) in dry DMF (2 ml) under an atmosphere of nitrogen. Stirring was continued at room temperature for 16 h, after which time, the crude reaction mixture was partitioned between ethyl acetate and brine. The separated organic layer was successively washed with 10% aqueous citric acid solution, saturated aqueous NaHCO_3 solution and brine before being dried (MgSO_4), filtered and concentrated under reduced pressure to afford compound 6 as a viscous oil.

Mass Spectrum (APCI) m/z 1226 [(M+H)⁺, 65%].

A solution of compound 6 (258 mg, 0.21 mmol) in dichloromethane (4 ml) was treated with trifluoroacetic acid (0.1 ml) under an atmosphere of nitrogen and stirring
5 was continued at room temperature for 20 h. The crude reaction mixture was concentrated under reduced pressure to afford a light tan oil which was purified by flash chromatography (silica, 6% methanol/dichloromethane elution). Concentration of the relevant fractions (R_f
10 0.30) afforded BRI7160 as a pale yellow foam.

Mass Spectrum (APCI) m/z 633 [(M-H), 100%].

A solution of BRI7160 (41 mg, 0.044 mmol) and Ni(OAc)₂·4H₂O (103 mg, 0.41 mmol) in dry methanol (2 ml) was heated at reflux for 19 h under an atmosphere of
15 nitrogen. The cooled reaction mixture was concentrated under reduced pressure to afford a solid residue. This material was treated with chloroform (4 ml) and the resulting suspension was filtered. Concentration of the filtrate afforded BRI7161 as a foam-like residue.

20 Mass Spectrum (APCI) m/z 1020 [(M+H)⁺, 30%].

Example 5 Testing of compound KJB030 for ability to inhibit amyloid β -peptide neurotoxicity in vitro

Compound KJB030 was dissolved in chelex 100-treated
25 double distilled H₂O (CH100-dH₂O) at a concentration of 200 μ M. A β 1-42 was dissolved in either 200 μ M KJB030 solution or in CH100-dH₂O. Both solutions contained 200 μ M A β 1-42. The A β 1-42 solutions were incubated at ambient temperature (~20-24°C) for 3 days. The solutions were then brought to a
30 physiological state by adding 10x phosphate buffered saline (PBS), pH 7.4, thus bringing samples to 1x PBS. Samples were then incubated at 37°C for 24 hr to induce aggregation of A β 1-42. Samples were subsequently dialysed in microdialysis cups (3500 M_r cut-off) placed in 5 L PBS for
35 24 hr, in order to remove some of the excess KJB030 without loss of A β 1-42.

Dialyzed samples were added to six day old cerebral cortical neuron cultures growing in Neurobasal medium with B27 supplements but without anti-oxidants (Gibco). Samples were diluted into the culture medium to a final

5 concentration of 20 μ M A β 1-42. The concentration of the KJB030 was unknown, due to dialysis of the sample; however, the maximum concentration would be 20 μ M if no dialysis had occurred. Six wells of each of the following were established: 1; Untreated control cells, 2; A β 1-42 (20 μ M),

10 3; KJB030 (dialysed), 4; KJB030+A β 1-42 (20 μ M, dialysed), 5; vehicle only (PBS). Cultures were treated for 5 days (37°C/5%CO₂) and culture medium was removed for analysis of cell death.

Determination of cell death was performed using the

15 lactate dehydrogenase (LDH) assay kit (Boehringer Mannheim). This is a measure of the release of LDH, a constitutively expressed, highly abundant enzyme, from dead (lysed) cells. Culture medium was mixed with the reagents from the LDH kit as per the manufacturer's instructions and

20 the level of LDH measured colourimetrically on a spectrophotometric 96 well plate reader at 490 nm. Using the equation supplied with the LDH assay kit, the percentage of LDH release and hence cell death compared to untreated control cultures was determined. The results are

25 shown in Table 1.

Table 1

Results of LDH assay for cell death

Treatment:	% cell death
Untreated control neurons:	0
Vehicle alone (PBS)	-1.18 \pm 1.4
KJB030 alone	8.1 \pm 2.2
A β 1-42 (20 μ M) alone	22 \pm 3.5
A β 1-42 (20 μ M) + KJB030	3.9 \pm 2.5*

*Significantly different from A β 1-42 alone (p<0.01)

Example 6 Demonstration of Reaction of Compound
KJB001 with A β 1-28

1.8 mgs of A β 1-28 was dissolved in 5 mls DMSO was
5 mixed with 0.33 mgs of compound KJB001 (~1 equivalent)
dissolved in 0.3 mls DMSO. The mixture was allowed to
stand for 24 hrs before being freeze-dried. The resulting
blue powder was dissolved in 550 μ l of an aqueous solution
containing 100 mM NaCl, 50 mM phosphate buffer, pH 6.9. A
10 solution of of 1.8 mgs A β 1-28 dissolved in 550 μ l of the
same solution was used as a control.

Figure 9(A) shows the 600 MHz ^1H NMR spectrum of the
aqueous solution of A β 1-28 at 271 K. The resonances due to
the C2H protons of His6, 13, 14 are marked with *. Figure
15 9(B) shows the ^1H 600 MHz NMR spectrum of the solution of
A β 1-28 plus KJB001, demonstrating that the peaks due to the
histidine C2H protons had shifted, thus indicating that
KJB001 had reacted with these residues.

20 DISCUSSION

When copper and iron bind to A β , reactive oxygen
species such as peroxide and superoxide are produced. When
copper and zinc bind to A β , both induce aggregation, and
copper binding is inhibited by zinc, suggesting that these
25 ions bind to similar binding sites, or share a single
binding site. Zinc, and presumably copper, bind to the
histidine residues of A β . Thus a molecule which prevents
the binding of zinc and copper to these histidine residues
has the potential to inhibit A β aggregation and to prevent
30 metal-induced neurotoxicity.

Compounds of the kind described herein have the
potential to bind to histidine residues and therefore to
prevent zinc and copper binding, and so may have
therapeutic value. A model of a cobalt-corrin ring complex
35 bound to A β 1-40 is shown in Figure 8.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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wherein the core molecule has a conformation and polarity such that the acid group(s) interact with one of more of His6, His13 and His14.

10. A compound according to claim 9, which is a molecule with one to three carboxylic acid groups, the length of the molecule being such that it can be received within the N-terminal loop, and such that at least one carboxyl group is in proximity to at least one of the histidine residues.

12. A compound according to claim 9, which is not a metal complex.

14. A compound according to claim 10, which is able to penetrate the blood-brain barrier.

16. A compound according to claim 15, in which the targeting moiety is selected from the group consisting of polypeptides, nucleic acids, carbohydrates, lipids, β -amyloid ligands, antibodies, and dyes.

18. A compound according to claim 17, in which the targeting moiety comprises a fatty acid molecule.

20. A compound according to claim 17, in which the targeting moiety is a peptide which comprises a sequence which corresponds to that of residues 15-21 of the β -amyloid peptide.

21. A compound according to any one of claims 15 to 20, in which the inhibitor-targeting moiety complex is able to penetrate the blood-brain barrier.

5 22. A method of selecting or designing a compound which inhibits the binding of metal ions to the N-terminal loop of the β -amyloid peptide, which method comprises the steps of

(i) selecting or designing a compound which has a
10 conformation and polarity such that it binds to at least
one amino acid in the N-terminal loop selected from the
group consisting of His6, His 13 and His14; and

(ii) testing the compound for the ability to inhibit binding of metal ions to the N-terminal loop of the β -amyloid peptide.

23. A method according to claim 22, in which the compound binds to at least two histidine residues in the N-terminal loop.

24. A method according to claim 23, in which the compound
20 binds to at least three histidine residues in the
N-terminal loop.

25. A method according to any one of claims 22 to 24, in which the compound also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11.

26. A method according to claim 26, in which the compound inhibits binding of Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.

27. A method according to any one of claims 22 to 26, in
30 which the compound has overall hydrophobic character.

28. A method according to claim 27, in which the compound is able to penetrate the blood-brain barrier.

29. A compound which inhibits the binding of metal ions to the N-terminal loop of the β -amyloid peptide, wherein

the compound is obtained by a method according to any one of claims 22 to 28.

30. A composition comprising a compound according to any one of claims 1 to 21 or claim 29, together with a pharmaceutically acceptable carrier.

31. A method of inhibiting the binding of one or more metal ions to the β -amyloid peptide, or of inhibiting the aggregation of β -amyloid peptide, which method comprises the step of exposing the peptide to a compound which blocks or destabilises the N-terminal loop of the peptide, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop.

32. A method according to claim 31, in which the compound has a conformation and polarity such that it binds to at least one histidine residue in the N-terminal loop of the β -amyloid peptide, selected from the group consisting of His6, His13 and His14.

33. A method according to claim 32, in which the
compound binds to at least two histidine residues in the
20 N-terminal loop.

34. A method according to claim 33, in which the compound binds to at least three histidine residues in the N-terminal loop.

35. A method according to any one of claims 31 to
25 34, in which the compound also binds to at least one
additional amino acid in the N-terminal loop, selected from
the group consisting of Asp7, Tyr10, and Glu11.

36. A method according to any one of claims 31 to 35, in which the compound inhibits binding of Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.

37. A method according to any one of claims 31 to 36, in which the compound is a complex of Mn, Fe, Co, Ni, Cu, Zn, Ru, Pd, Ag, Cd, Pt, Au, Rh or Hg, with the proviso that the compound is not haemin or haematin.

35 38. A method according to any one of claims 31 to 37, in
which the compound comprises, or is conjugated to, a
targeting moiety.

39. A method according to claim 38, in which the targeting moiety targets the compound to the site defined by residues 15-21 on the β -amyloid peptide.

5 40. A method according to any one of claims 31 to 39, in which the inhibition of binding of one or more metal ions to the β -amyloid peptide occurs *in vivo*.

41. A method of prevention, treatment or alleviation of Alzheimer's disease, which method comprises the step of administering a compound according to any one of claims 1
10 to 21 or a pharmaceutical composition according to claim 30 to a subject in need of such treatment.

42. A method of prevention, treatment or alleviation of Alzheimer's disease, which method comprises inhibiting the binding of one or more metal ions to the β -amyloid peptide,
15 or inhibiting the aggregation of β -amyloid peptide, by the method of claim 40.

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(54) Title: BETA-AMYLOID PEPTIDE INHIBITORS

(57) Abstract: The present invention relates to compounds which inhibit the binding of metal ions to a region in the N-terminal loop of the β -amyloid peptide which includes a cluster of histidine residues. In addition, the invention relates to pharmaceutical compositions including these compounds as the active agent, and to methods of treatment involving the administration of these compounds. The compounds of the invention are useful in the treatment of Alzheimer's disease and other amyloid-related conditions. In a first aspect the present invention provides a compound which interacts with the β -amyloid peptide in such a way that the N-terminal loop of the peptide (amino acid residues 1-15) is blocked or destabilised, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop. Preferably the compound inhibits binding of Cu^{2+} , Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.

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Figure 1

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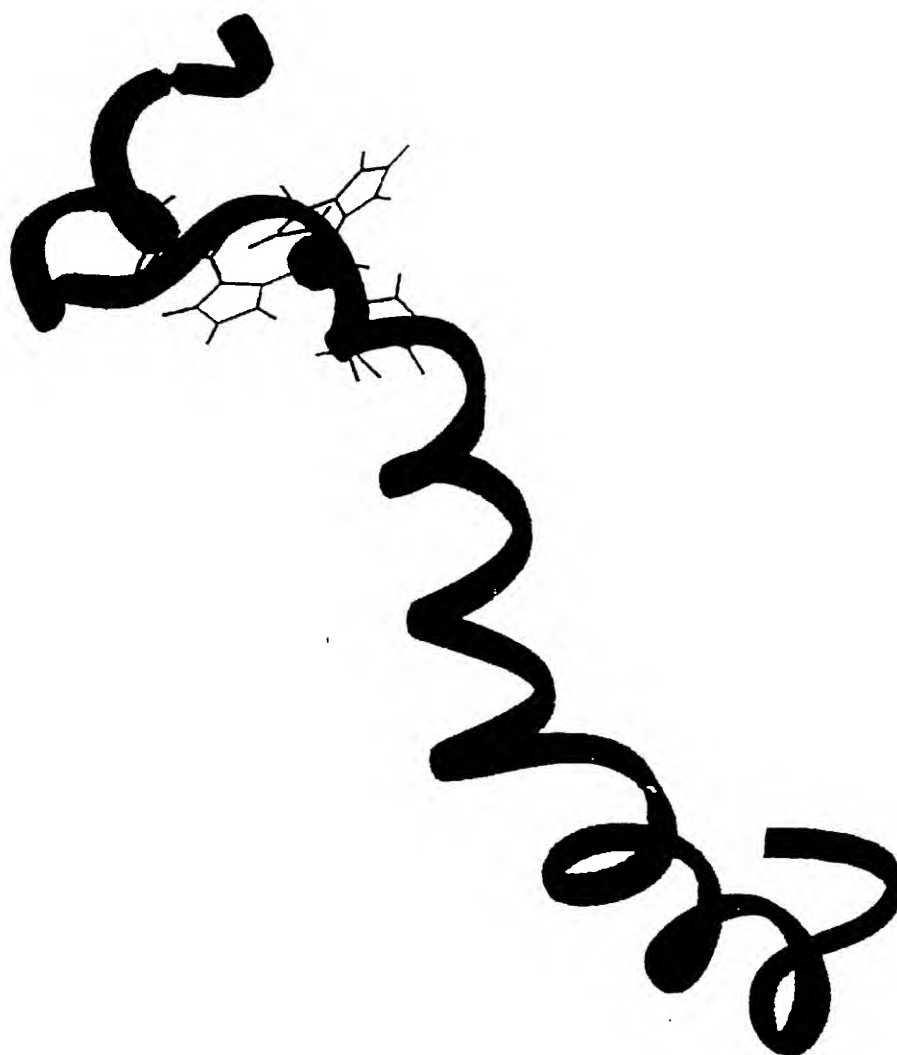


Figure 2

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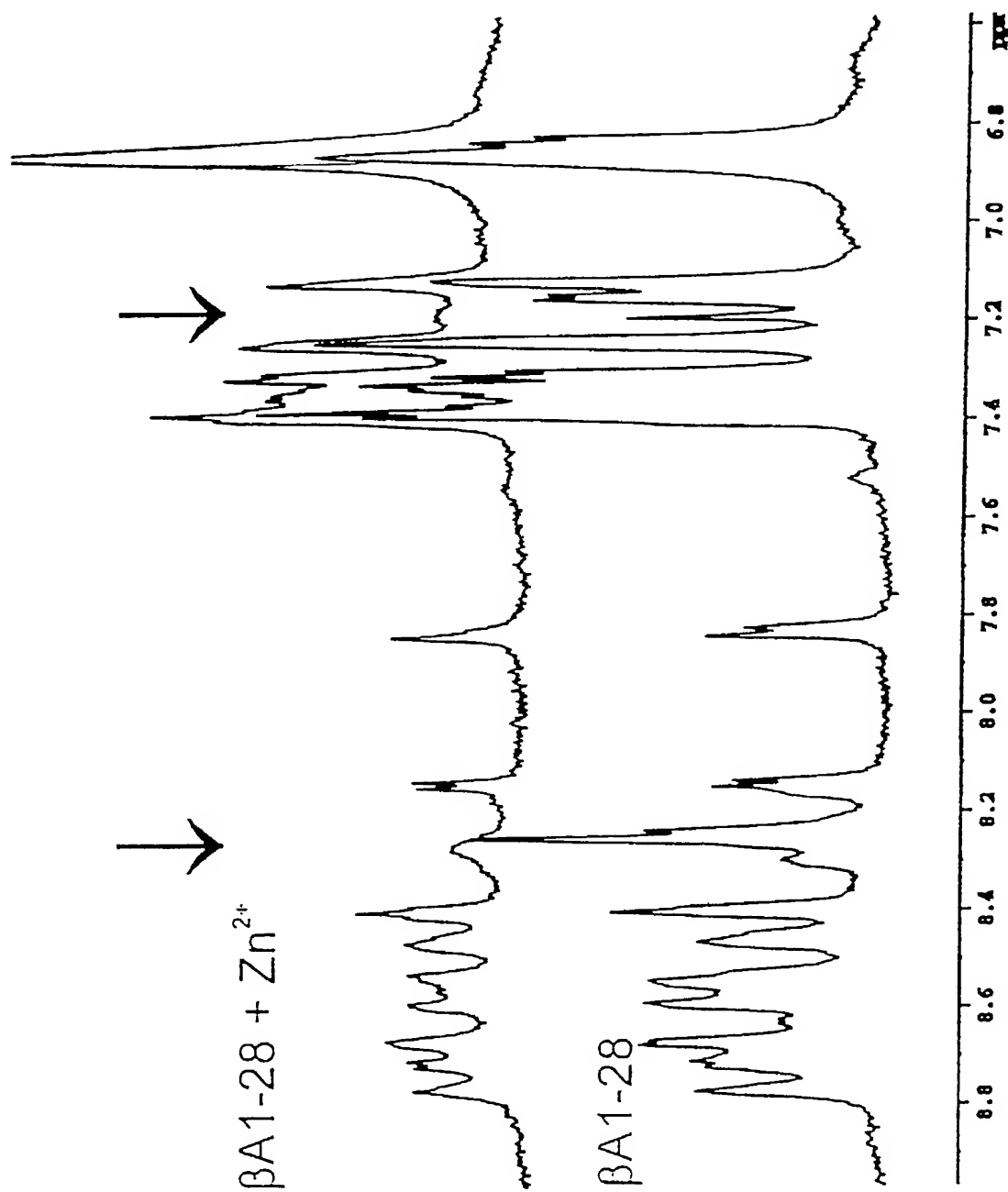
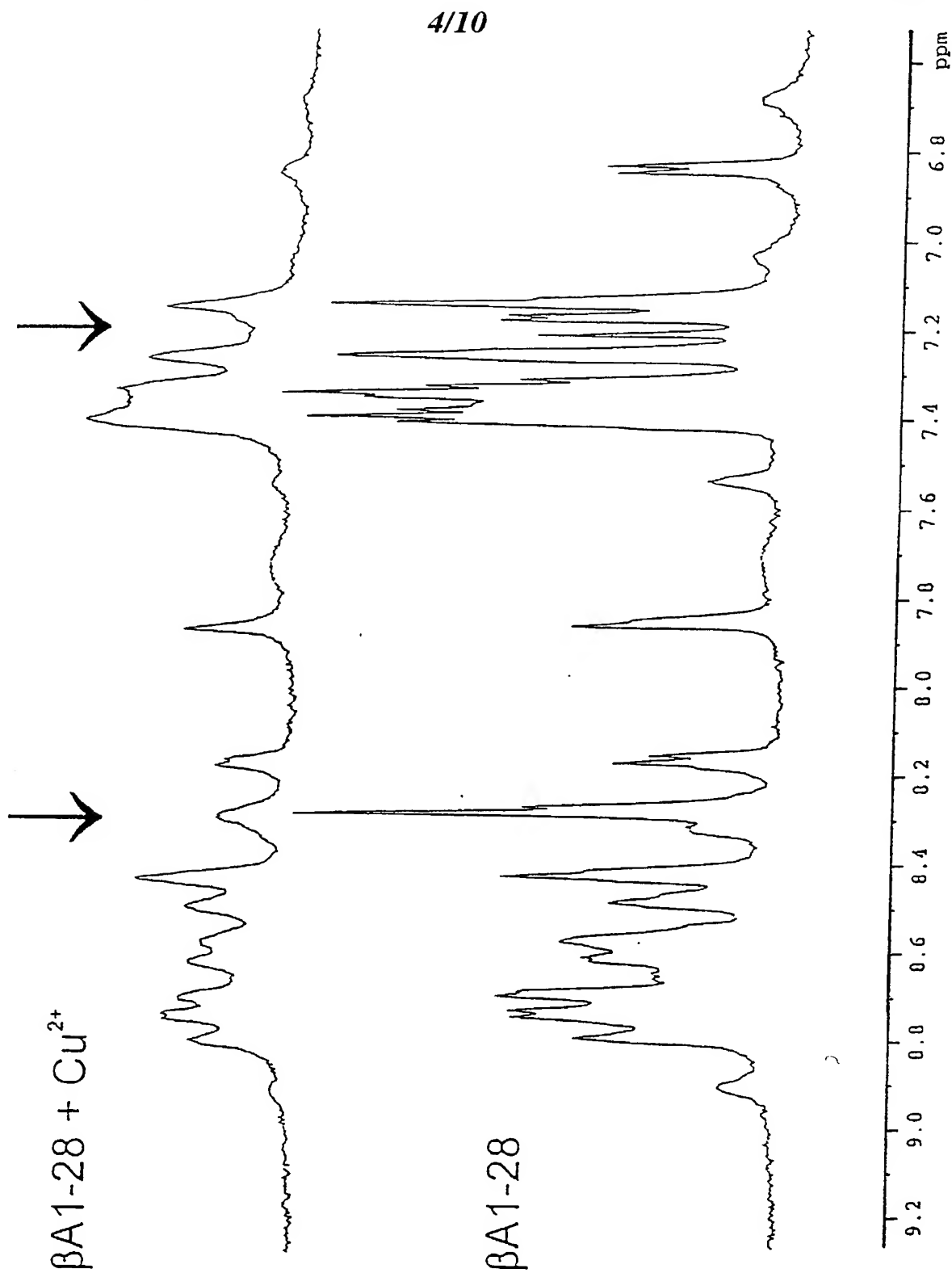
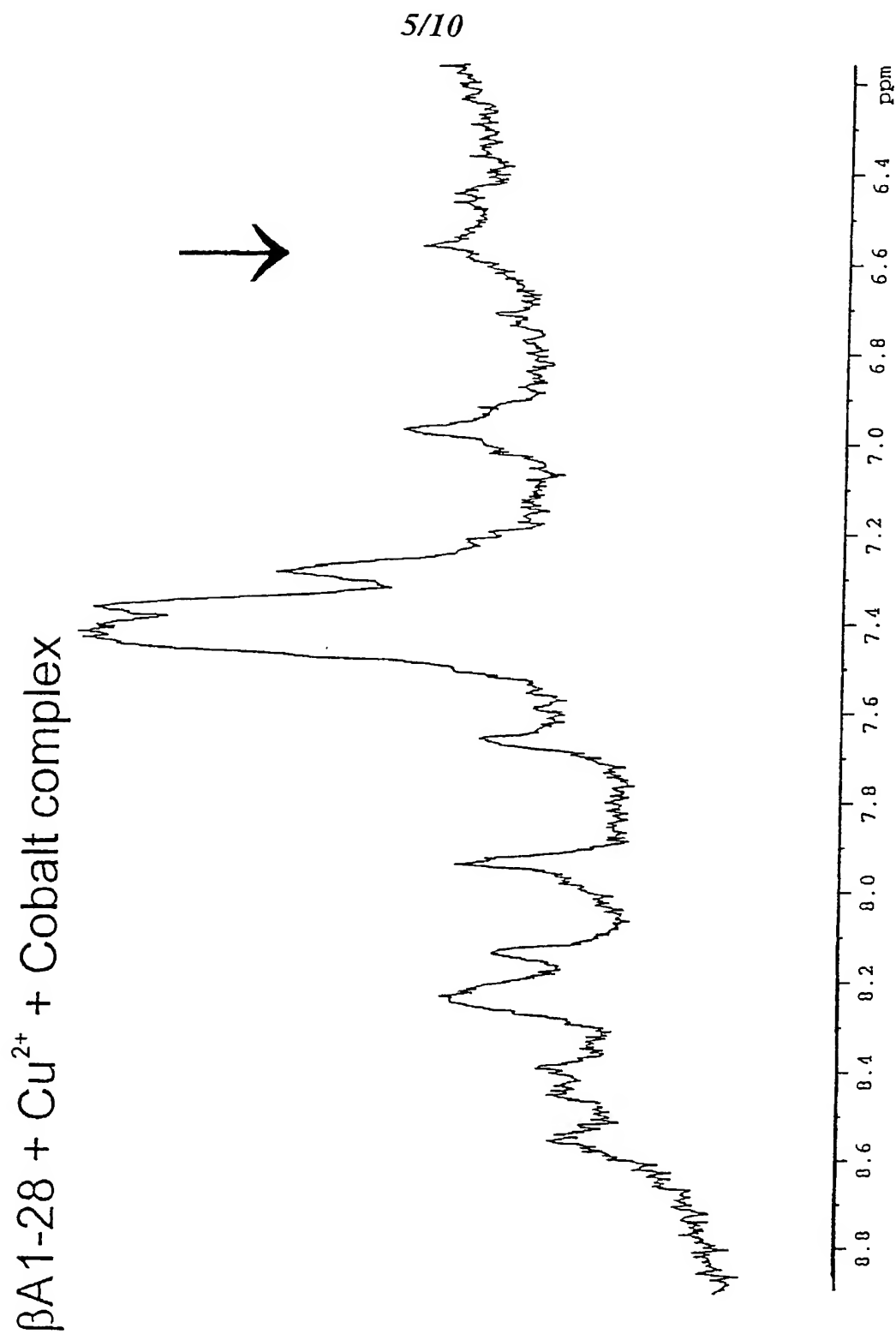


Figure 3

*Figure 4*

*Figure 5*

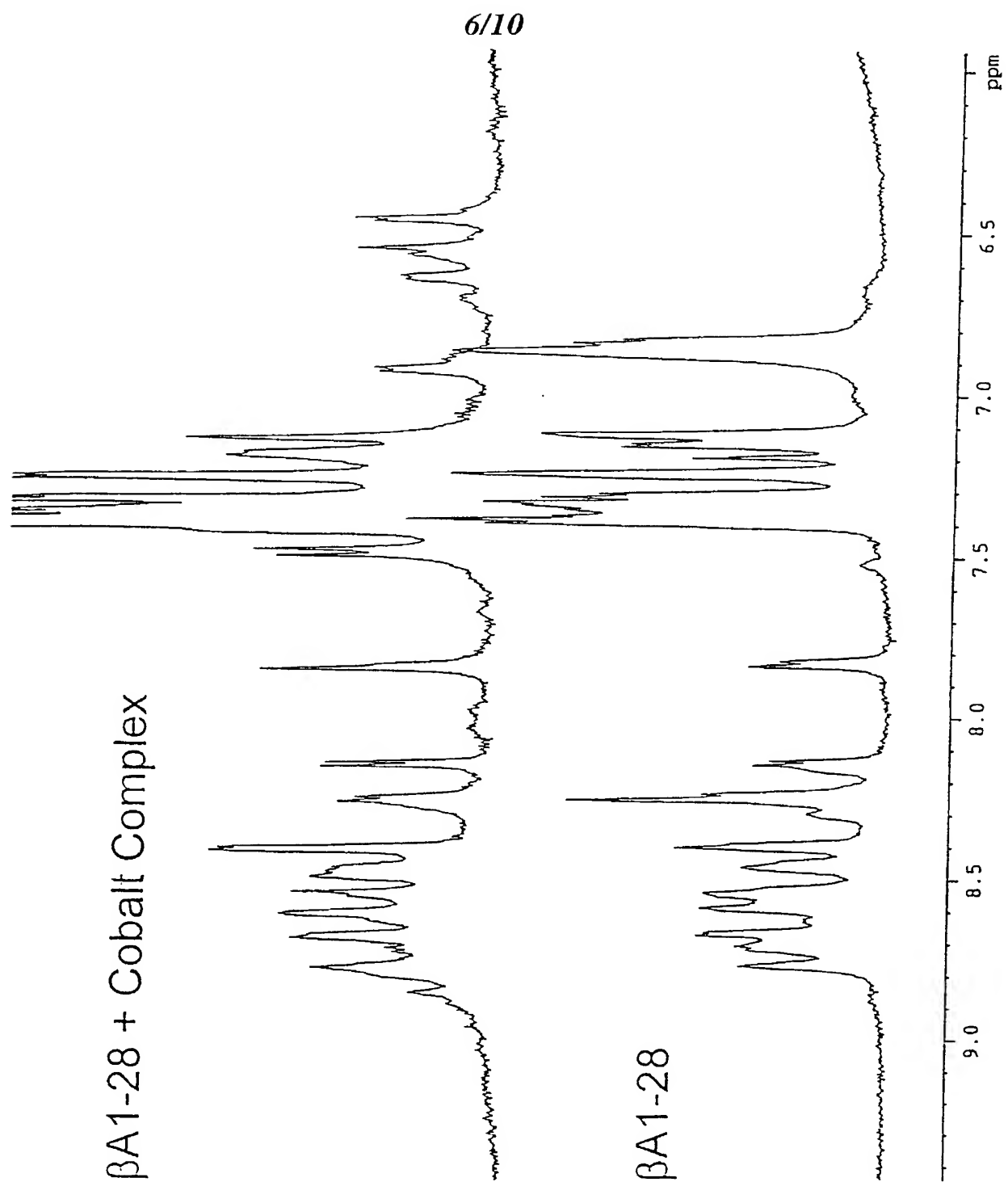
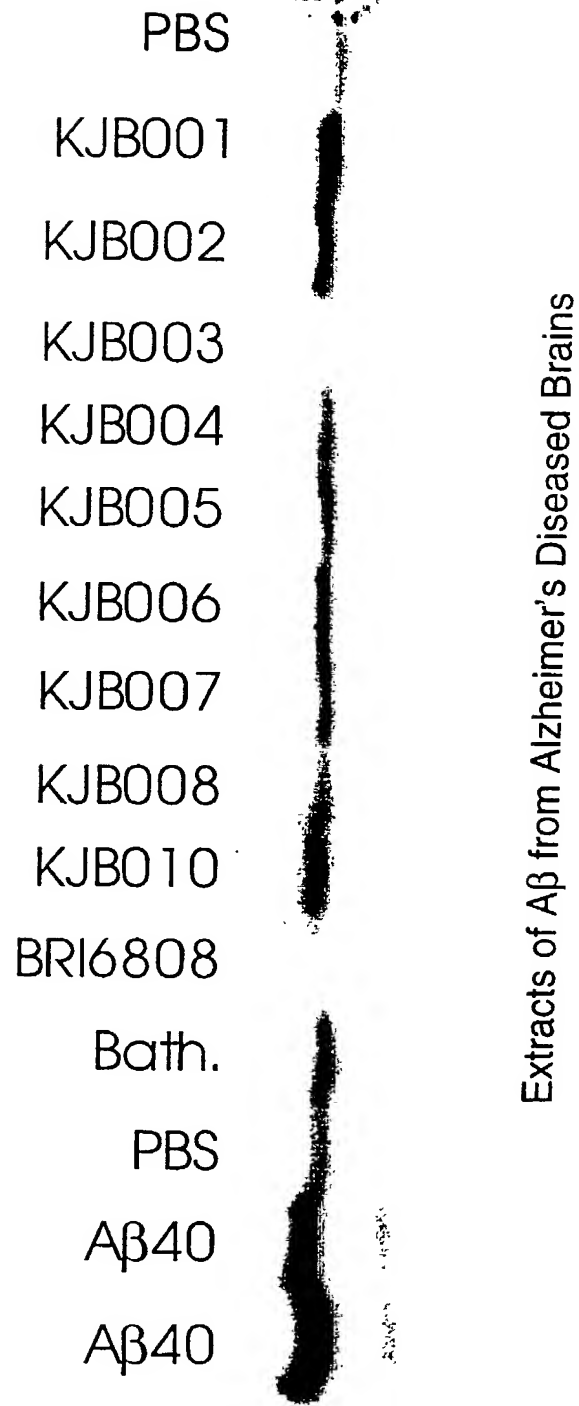


Figure 6

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**Figure 7**

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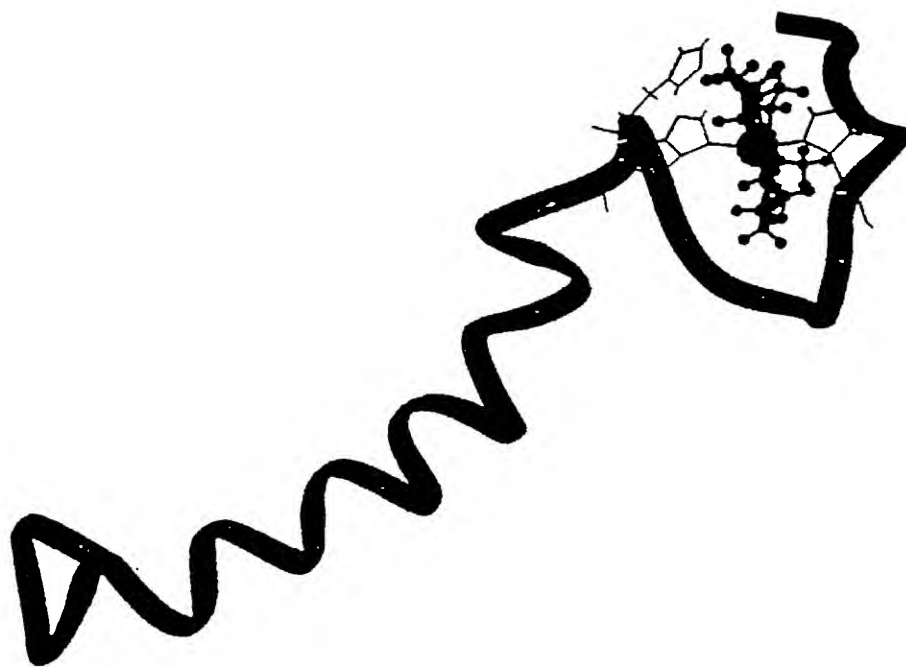


Figure 8

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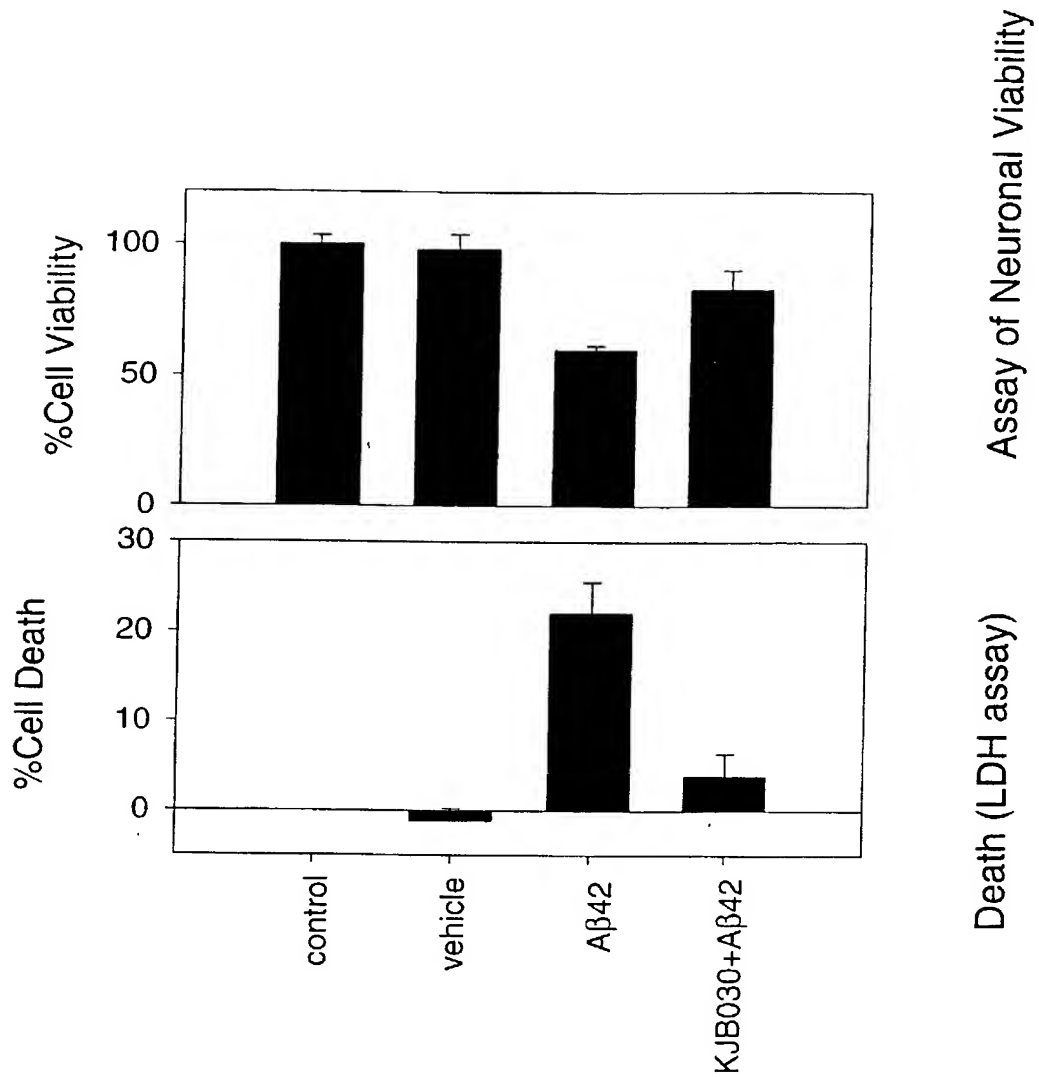
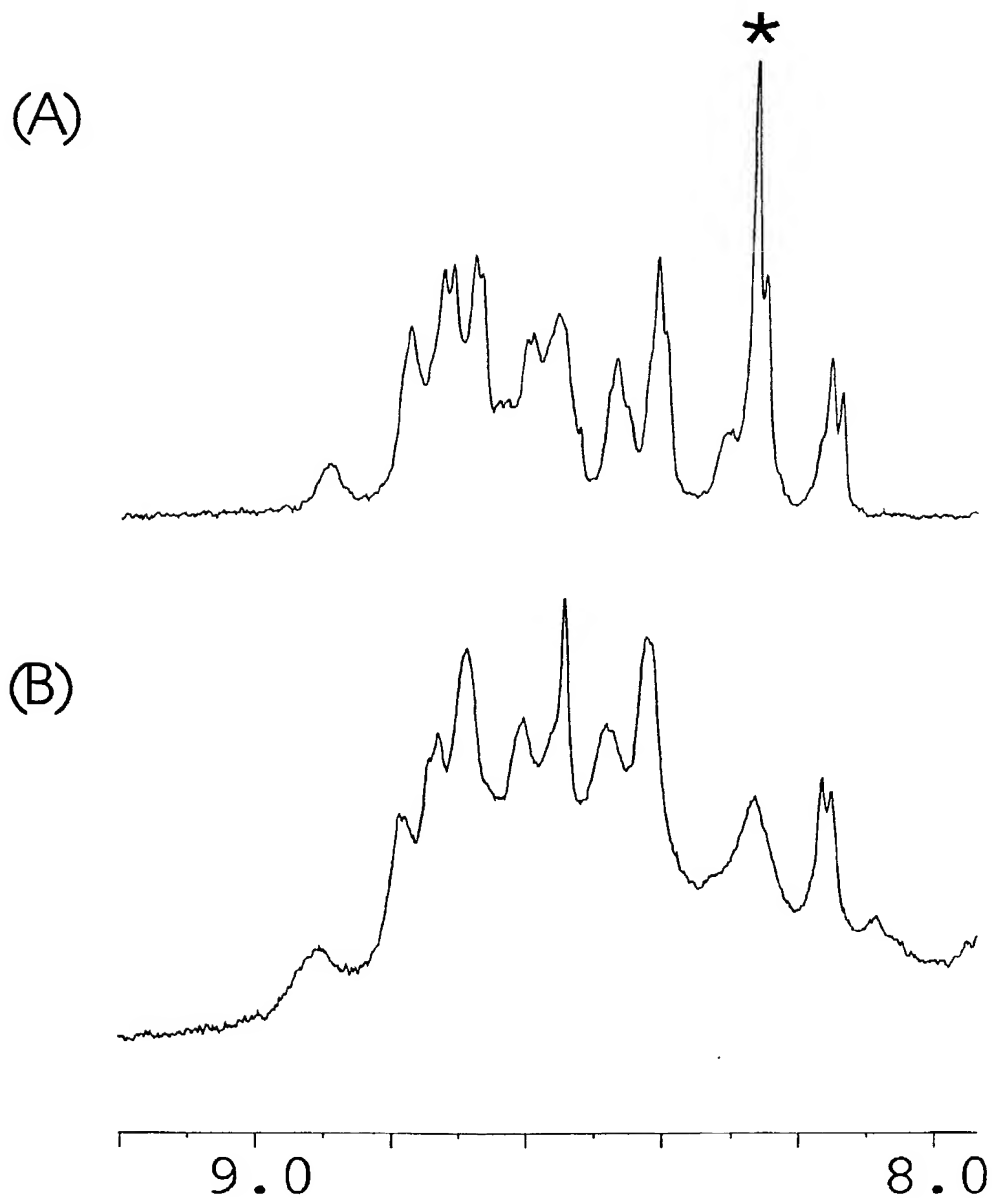


Figure 9

10/10

*Figure 10*

DECLARATION AND POWER OF ATTORNEY
(Attorney Docket No: 113122.120)

As below-named inventors, We hereby declare that:

Our residences, post office addresses and citizenship are as stated below next to our names.

We believe that we are the original, and only inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled:

BETA-AMYLOID PEPTIDE INHIBITORS

the specification of which (check only one):

- ☐ is attached hereto.
- ☒ was filed as United States Patent Application
Serial No. 10/031,478
on January 18, 2002 (International Filing Date: July 21, 2000)
and was amended on _____
(if applicable)
- ☐ was filed as PCT Patent Application
Serial No. _____
on _____
and was amended under PCT Article 19
on _____
(if applicable)

We hereby state that we have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the patentability of the claims of this application in accordance with Title 37, CFR §1.56(a) and §1.56(b). We also acknowledge the duty to disclose all information which is material to the patentability as defined in 37 CFR §1.56, which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

We hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate or 365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by us on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS
UNDER 35 U.S.C. §119(a)-(d) or 365(b), or 365(a):

COUNTRY (if PCT indicate PCT)	APPLICATION NUMBER	DATE OF FILING	PRIORITY CLAIMED UNDER 35 U.S.C. §119 (YES/NO)
PCT	PCT/AU00/00886	July 21, 2000	Yes
AU	PQ 1804	July 23, 1999	Yes

POWER OF ATTORNEY: As named inventors, We hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Michael A. Diener	Reg. No. 31,122
Colleen Superko	Reg. No. 39,850
Richard A. Goldenberg	Reg. No. 38,895
Peter M. Dichiaro	Reg. No. 38,005
Barbara A. Barakat	Reg. No. 32,190
Jason A. Reyes	Reg. No. 41,513
Rajesh Vallabh	Reg. No. 35,761
Nancy Chiu, Ph.D.	Reg. No. 43,545
Keum J. Park	Reg. No. 42,059
Janice M. Klunder, Ph.D.	Reg. No. 41,121
Sally Byrne	Reg. No. 40,545
Ayla A. Lari	Reg. No. 43,739
Nels Lippert	Reg. No. 25,888
Robert McIsaac, Ph.D.	Reg. No. 46,918
Gretchen A. Rice, Ph.D.	Reg. No. 37,429
David L. Cavanaugh	Reg. No. 36,476
Joseph F. Haag	Reg. No. 42,612
Wendy A. Haller	Reg. No. 35,177
MaryRose Scozzafava, Ph.D.	Reg. No. 36,268
Michael J. Twomey	Reg. No. 38,349
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60 State Street
Boston, Massachusetts 02109

(617) 526-6000
(617) 526-5000

Wherefore we petition that letters patent be granted to us for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe our names to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: Kevin Jeffrey Barnham

1st Inventor's signature _____ Date _____
Residence: Brunswick Road, West Brunswick, VIC 3055, Australia
Citizenship: Australia
Post Office Address: same as residence

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Full name of second inventor: Thomas David McCarthy

2nd Inventor's signature _____ Date _____
Residence: 40 Tooronga Road, Malvern East, VIC 3145, Australia
Citizenship: Australia
Post Office Address: same as residence

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Full name of third inventor: Susanne Pallich

3rd Inventor's signature Spallich X Date 13/6/02 X
Residence: 17 Poole Street, Deer Park, VIC 3023, Australia
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Full name of first inventor: **Kevin Jeffrey Barnham**

Inventor's signature

Date

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Full name of second inventor: **Thomas David McCarthy**

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Full name of third inventor: **Susanne Pallich**

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Date

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Full name of fourth inventor: **Barry Ross Matthews**


Inventor's signature _____ Date _____

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Full name of fifth inventor: **Robert Alan Cherny**

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